The Dissertation Committee for Lisa Ferguson Stegall Certifies that this is the approved version of the following dissertation:

THE EFFECTS OF CARBOHYDRATE-PROTEIN SUPPLEMENTATION ON ENDURANCE EXERCISE PERFORMANCE, RECOVERY, AND TRAINING ADAPTATION

Committee:

John L Ivy, Supervisor

Nomeli P. Nunez

Hirofumi Tanaka

Lisa Griffin

Christopher Jolly

THE EFFECTS OF CARBOHYDRATE-PROTEIN SUPPLEMENTATION ON ENDURANCE EXERCISE PERFORMANCE, RECOVERY, AND TRAINING ADAPTATION

by

Lisa Ferguson Stegall, B.A., M.S.

Dissertation

Presented to the Faculty of the Graduate School of

The University of Texas at Austin

in Partial Fulfillment

of the Requirements

for the Degree of

Doctor of Philosophy

The University of Texas at Austin

December, 2010

Acknowledgements

I extend my heartfelt gratitude to the following people, without whom this dissertation would have not been possible (nor would the last 4.5 years been as much fun):

First, I thank Dr. John Ivy for taking on this one “last” Ph.D. student, and for his priceless guidance in my development as a future independent scientist. My gratitude goes to Zhenping Ding for sharing his wealth of biochemical knowledge, but most of all, for his unwavering patience, quiet encouragement, and genuine kindness. I also thank my committee members for their support: Dr. Tanaka, Dr. Griffin, Dr. Jolly, and Dr. Nunez.

Special appreciation goes to Dr. Ed Coyle for being my first advisor at UT, and for remaining my mentor and friend ever after. My gratitude also goes to Dr. Roger Farrar for always taking the time to fulfill his valuable role as giver of wisdom and advice.

Studies 2, 3, and 4 would have been impossible without the dedication of Erin McCleave and Phil Doerner, and it would have been far less cheerful at o-dark-thirty without them to work with all those months. In addition, I send out special thanks to Erin for all those Crown and Anchor breaks that helped keep us smiling through the exhaustion. I sincerely thank my past and present Exercise Physiology and Metabolism Lab mates who provided their time and energy to help with these studies; without the efforts of everyone in the lab, these studies would not have been so successful.

I can’t say thanks enough to my dear friends Ed, Matt, and Jill for the countless coffee breaks across the street, fun social outings, and gossip sessions in the hallway. (We were really discussing science all those times, really!) They are a part of special memories from weddings (mine and a couple of theirs), to parties, to concerts, lots of pub nights and gory mountain bike disasters. In the mosaic of memories that comprise the graduate school years, these guys added lots of color and light.

I also say a warm thank you to my dear friend Erica McKenzie, who has been a tremendous source of encouragement and a wonderful friend for many years.

A special thank you goes to Yang Liu for helping with my studies tirelessly and with inspiring dedication, and for giving me the opportunity to be a mentor to such a promising student.

I wholeheartedly thank my supportive and loving husband, David, for putting up with me through this process, for listening to my silly rants, discussing my research ideas with me, and celebrating my little moments of victory over things like tricky Western blots. Most of all, I thank him for being the wonderful person that I love.

I also express deep gratitude to my parents, Noel and Eloise, whose genes, love, countless sacrifices, encouragement, and belief in their little daughter's abilities gave me the foundation of confidence and "stick-to-it-ness" that helped to carry me through this journey. I thank them for their example and their pride in me.

I would also like to acknowledge the dedicated efforts of the subjects that volunteered for these studies, giving up precious time, sweat, blood and muscle tissue for the sake of science, and in many cases, as a token of friendship.

Finally, I would like to thank HPL Laboratories (Study 1) and the National Dairy Council and National Fluid Milk Processor Promotion Board (Studies 2, 3 and 4) for funding my research.

THE EFFECTS OF CARBOHYDRATE-PROTEIN SUPPLEMENTATION ON ENDURANCE EXERCISE PERFORMANCE, RECOVERY, AND TRAINING ADAPTATION

Lisa Ferguson Stegall, Ph.D.

The University of Texas at Austin, 2010

Supervisor: John Ivy

Recent research suggests that adding protein (PRO) to a carbohydrate (CHO) supplement can have substantial benefits for endurance exercise performance and recovery beyond that of CHO alone. CHO+PRO supplements are often commercially available formulations consisting of carbohydrates (dextrose, maltodextrin) and whey protein. The effects of a supplement containing moderate protein and a low-CHO mixture on endurance performance has not been investigated. Also, the effects of CHO+PRO supplementation in the form of a natural food, flavored milk, on measures of recovery from acute endurance exercise, as well as on chronic aerobic exercise training adaptations, have not been characterized. Therefore, in this series of four studies, the effects of CHO+PRO supplementation on the following areas of endurance exercise performance, recovery, and adaptation are investigated: acute endurance exercise performance, inflammatory and muscle damage markers, muscle glycogen resynthesis, activation of signaling proteins involved in the initiation of protein synthesis and degradation, subsequent endurance exercise performance, and chronic aerobic training adaptations (maximal oxygen consumption, oxidative enzyme activity, body composition, immune cell levels, and inflammatory markers). Study 1 demonstrated that

a supplement containing a low-CHO mixture plus moderate protein significantly improved aerobic endurance when cycling at or below the ventilatory threshold, despite containing 50% less CHO and 30% fewer calories relative to a higher CHO beverage. Study 2 demonstrated that CHO+PRO supplementation in the form of chocolate milk (CM) is an effective post-exercise supplement that can improve subsequent performance and provide a greater intracellular signaling stimulus for protein synthesis compared to CHO and placebo. Study 3 found that post-exercise CM supplementation during 4.5 wks of aerobic exercise training improves the magnitude of cardiovascular adaptations more effectively than isocaloric CHO or placebo, while the fourth study demonstrated that post-exercise CM supplementation during 4.5 wks of aerobic training improves body composition more effectively than isocaloric CHO or placebo. The fourth study also demonstrated that 4.5 wks of training does not appear to perturb resting immune cell concentrations or markers of inflammation and muscle damage. Taken together, the results of this research series suggest that CHO+PRO supplementation extends endurance performance, improves recovery, and increases training adaptations more effectively than CHO or placebo.

Table of Contents

List of Tables

List of Figures

Chapter I: General Introduction

Proper nutritional supplementation during endurance exercise is essential for delaying fatigue and maintaining optimal performance. Providing the right nutrients following intense exercise is also critical, as adequately recovering from exhaustive endurance exercise is central to the ability to perform at one's best day after day in training sessions and competitive events. Recovery is also essential for achieving positive training adaptations that occur in response to such exercise. It is well known that immediately after prolonged, intense endurance exercise, a catabolic physiological state prevails: catecholamines and cytokines are elevated, blood glucose and insulin levels are low, muscle and liver glycogen stores are depleted and immune function is suppressed. As such, the body's ability to perform such exercise again in a matter of hours or even days is compromised, as is the ability to adapt to the training stimuli for long-term endurance performance improvements.

In order to shift from a catabolic to an anabolic physiological state post-exercise, optimal nutrients must be provided. Replenishing depleted glycogen stores is of primary emphasis, as prolonged, intense exercise simply cannot be performed without adequate glycogen stores. Despite its great importance, glycogen is not the only variable central to recovery that is modulated by optimal post-exercise nutrition. Endurance exercise recovery is a highly complex process that involves the integration of multiple systems and many components, including immune system function, tissue damage repair, and protein synthesis pathways. All of these are positively affected by proper post-exercise nutrition.

Eating or drinking something simply containing calories following endurance exercise is not enough, however, to shift into an optimally anabolic state. It is important to consume the appropriate amounts and types of nutrients post-exercise, and with appropriate timing. When nutrient supplementation is performed properly and strategically, recovery from exercise is significantly enhanced, immune function is protected, subsequent endurance exercise performance is improved, and training

adaptations are increased.

It is well known that carbohydrate is essential for recovery from prolonged, strenuous endurance exercise. However, recent research suggests that the addition of protein (PRO) to a carbohydrate (CHO) supplement can have substantial benefits beyond that of providing CHO alone. Several investigators have found that post-exercise CHO+PRO supplementation will speed the recovery process and improve subsequent exercise performance (2, 6-7, 9-10).

Therefore, this series of studies aimed to investigate the effects of CHO+PRO supplementation on the following key areas of endurance exercise performance, recovery and adaptation: acute endurance exercise performance to exhaustion, muscle glycogen resynthesis, markers of inflammation, muscle damage and protein degradation, activation of signaling proteins involved in the initiation of protein synthesis, subsequent endurance exercise performance, and training adaptations (specifically, maximal oxygen consumption, muscle oxidative enzyme activity, body composition, and immune cell levels).

OBJECTIVES

Study 1: to determine if a low-CHO + moderate PRO supplement given during endurance exercise can increase time to fatigue and reduce muscle damage that occurs during strenuous endurance exercise, despite containing 50% less carbohydrate and fewer calories than a CHO supplement.

Study 2: to determine if muscle glycogen resynthesis following intense endurance exercise is increased with a CHO+PRO beverage in the form of chocolate milk (CM) given post-exercise, as compared to a CHO-only beverage or placebo, and to then determine if subsequent endurance exercise performance is improved after the recovery period. In addition, we purposed to determine if indicators of post-exercise inflammation, muscle damage and degradation is reduced in the CM treatment compared to the CHO or placebo treatments, and also determine the effects of the experimental treatments on the activation states of signaling proteins that control translation initiation for protein synthesis.

Study 3: to determine if supplementing with a CHO+PRO beverage post-exercise compared to CHO only or placebo over a 4.5-week period of intense endurance exercise training results in a greater increase in maximal oxygen uptake, muscle oxidative enzyme activity, and indicators of mitochondrial biogenesis.

Study 4: to determine if supplementing with a CHO+PRO beverage post-exercise compared to CHO only or placebo over a 4.5-week period of intense endurance exercise training results in a greater improvements in body composition in the CHO+PRO group compared to the CHO or placebo groups. We also aimed to determine if immune cell levels are less perturbed, and markers of inflammation and muscle damage better attenuated, in the CHO+PRO group compared to the CHO or placebo groups.

HYPOTHESES

Study 1

1. In comparison with a CHO-only supplement, a CHO+PRO supplement containing a lower amount of carbohydrate and a moderate amount of protein (MCP) taken during exercise will extend time to fatigue during strenuous cycling endurance exercise despite containing 50% less carbohydrate and 30% fewer calories than the CHO beverge.

Study 2

1. In comparison with a CHO supplement and placebo, a dairy CHO+PRO supplement (chocolate milk, CM) will: (a) increase muscle glycogen resynthesis after a prolonged aerobic exercise bout that depletes the muscle glycogen stores, and (b) will enhance subsequent exercise performance, measured in the amount of time taken to complete a 40-km time trial.
2. In comparison with an isocaloric CHO supplement and a placebo treatment, a CHO+PRO supplement will demonstrate greater activation states of cellular signaling proteins that control mRNA translation initiation (mTOR, rpS6, and eIF2Bε), and less protein degradation as assessed by FOXO3A phosphorylation and total ubiquitination of muscle tissue samples.

3. In comparison with an isocaloric CHO supplement and Placebo, a CHO+PRO supplement will better promote exercise recovery by: (a) reducing protracted exercise muscle damage as assessed by myoglobin and CPK levels, and (b) will reduce inflammation as measured by pro- and anti-inflammatory cytokine levels during recovery.

Study 3

1. In comparison with an isocaloric CHO supplement and placebo, a CM supplement given post-exercise will (a) increase the rate of training adaptations, defined as increased VO_2max, increased muscle oxidative enzyme activity, and increase in an indicator of mitochondrial biogenesis, that occur over 4.5 weeks of endurance training (cycling).

Study 4

1. In comparison with an isocaloric CHO supplement and placebo, a CM supplement will demonstrate improved body composition (maintenance of, or increase in, lean muscle mass and reduction in fat mass) during periods of chronic intense endurance training.
2. In comparison with an isocaloric CHO supplement and placebo, a CM supplement will demonstrate less perturbations in circulating immune cell levels during periods of chronic intense endurance training.

SIGNIFICANCE

In the first study, we aimed to demonstrate the effectiveness of a low CHO + moderate PRO beverage in improving endurance performance and extending time to fatigue. A during-exercise supplement containing fewer total calories, yet an optimal blend of carbohydrate plus protein would fill a need for exercisers and athletes who desire a lower calorie but effective supplement and serve as an alternative to many of the traditional supplements.

Recent research suggests that a CHO+PRO supplement can have substantial benefits over CHO alone after exercise such that recovery is hastened and subsequent exercise performance is improved (2, 6-10). The recovery study described here is the first

to investigate all of these parameters in response to CHO+PRO supplementation in the form of flavored milk. Likewise, the training studies (Studies 3 and 4) will be the first to investigate the adaptive response to aerobic training with post-exercise CHO+PRO/CM supplementation compared to CHO and placebo. If we were able to show a significant improvement in recovery measures as well as training adaptation with CM supplementation, this could yield a viable, natural low-cost recovery supplement for the endurance athlete and exerciser.

Studies three and four examined the training adaptations that occur over a 4.5-week endurance exercise training period in untrained individuals. If we demonstrate an increased rate of adaptation and increased fitness gain (as evidenced by improved maximal oxygen consumption and muscle oxidative enzyme activity) in the group supplementing with CM, then this would provide powerful evidence upon which sports nutritionists and coaches can base recommendations for proper recovery supplementation to exercisers and athletes in order to maximize the adaptive response to the training stimulus. Demonstrating improvements in body composition would be a novel and important finding for those seeking to decrease fat mass while gaining lean tissue during aerobic exercise training.

CHO supplementation alone during prolonged exercise attenuates the typical increase in plasma stress hormones, such as cortisol, and potent pro-inflammatory cytokines such as IL-6 and TNFα, while lowering blood neutrophil and monocyte counts (3-5). Surprisingly, the potential for added benefits to be gained from adding protein to an exercise or recovery drink has not yet been investigated. Therefore, given that CHO supplementation alone significantly reduces some aspects of the decline in immune function in response to intense exercise stress, and given that CHO+PRO has many demonstrated beneficial effects on performance and recovery that supersede the effects of CHO alone, including reductions in muscle damage (1, 7), we also hypothesized in Study 4 that CM supplementation would attenuate immune cell and inflammatory marker perturbations in response to exercise training to a greater extent than CHO alone or placebo. This would be a significant finding, as reducing risk of infection and illness is

beneficial to the elite athlete, as well as to the avid exerciser who seeks to improve fitness without the risk of avoidable minor illnesses.

Taken together, the goals of these studies was to demonstrate more effective ways to improve endurance exercise performance as well as recovery from strenuous exercise, and to increase the magnitude of adaptation to exercise training, while also reducing muscle damage and maintaining immune function. Ideally, this would translate to improved fitness, reduced illness and infection risk, and improved overall health for the endurance athlete and exerciser.

LIMITATIONS AND DELIMITATIONS

Although every attempt is made to recruit subjects with similar fitness levels, it is possible that there was variation in those levels and in individual training plans that could contribute to differing responses to the experimental trials in the first two studies. Since variations in normal dietary status or physical activity levels could affect substrate metabolism, subjects in all studies were asked to consume the same diet for the two days preceding each trial during which blood or muscle tissue was taken, and keep their activity levels consistent for the 3 days prior as well, so that starting muscle glycogen and glucose levels were not influenced by dietary and/or physical activity variation. However, standardizing the diet and training across the entire study period would likely yield better consistency and eliminate the possibility for performance differences between subjects due to these possible variations.

Other possible limitations in these studies lie in techniques and measurement methods. When taking muscle biopsies, it is possible to not extract enough usable tissue to be able to accurately assess the signaling protein activation states or amounts of the oxidative enzymes. In addition, we use the standard technique for muscle glycogen determination where biopsies are taken from the vastus lateralis and the glycogen concentration is determined after its complete enzymatic degradation to glucose with amyloglucosidase. Liberated glucose is then measured using a spectophotometric Trinder reaction. While this method is used extensively in such studies, a newer, yet very expensive technique – NMR – is available that would allow for less invasiveness, more

frequent measurement time points, and greater accuracy. Due to funding limitations, this technique was beyond the scope of these studies.

The use of chocolate milk as the CHO+PRO supplement of choice in the recovery and training studies has limitations. It is likely that chocolate milk is digested quite differently from typical carbohydrate-protein supplements that simply contain 1-3 carbohydrates and whey protein isolate. Therefore, it would be useful in future studies to include a standard CHO+PRO treatment group in addition to the CM, CHO only, and placebo treatments to tease out the effects of the milk-based supplement. Although difficult to achieve, it would also be preferable to better disguise the non-CM treatments by either using chocolate flavored carbohydrate gels in solution, or using a cocoa+sugar power that lacks protein. This would reduce the subjects' perception of what treatment they are receiving and help to eliminate confounding psychological responses to the treatments.

The subjects in these studies were all in the age range of 18 – 39. Therefore, our results may not be generalizable to younger or older athletes. Also, given that the mode of exercise we studied is cycling, it is possible that the results, particularly concerning markers of muscle damage, may differ from what could be found in a more eccentric contraction-based mode, such as running or in resistance training. Therefore, the results may not be generalizable to other types of endurance athletes. In addition, the measures of body composition and plasma metabolic hormones in the training study are done in healthy untrained subjects, and the results may not be generalizable to populations such as individuals who are insulin resistant, or who are obese.

REFERENCES

1. Baty JJ, Hwang H, Ding Z, Bernard JR, Wang B, Kwon B, and Ivy JL. The effect of a carbohydrate and protein supplement on resistance exercise performance, hormonal response, and muscle damage. J Strength Cond Res 21: 321–329, 2007.
2. Ivy JL, Goforth HW, Jr., Damon BM, McCauley TR, Parsons EC, and Price TB. Early postexercise muscle glycogen recovery is enhanced with a carbohydrate-protein supplement. J Appl Physiol 93: 1337-1344, 2002.
3. Nieman DC, Davis JM, Henson DA, Walberg-Rankin J, Shute M, Dumke CL, Utter AC, Vinci DM, Carson JA, Brown A, Lee WJ, McAnulty SR, and McAnulty LS. Carbohydrate ingestion influences skeletal muscle cytokine mRNA and plasma cytokine levels after a 3-h run. J Appl Physiol 94: 1917-1925, 2003.
4. Nieman DC, Henson DA, Smith LL, Utter AC, Vinci DM, Davis JM, Kaminsky DE, and Shute M. Cytokine changes after a marathon race. J Appl Physiol 91: 109-114, 2001.
5. Nieman DC, Nehlsen-Cannarell SL, Fagoaga OR, Henson DA, Utter A, Davis JM, Williams F, and Butterworth DE. Influence of mode and carbohydrate on the cytokine response to heavy exertion. Med Sci Sports Exerc 30: 671-678, 1998.
6. Niles ES, Lachowetz T, Garfi J, Sullivan W, Smith JC, Leyh BP, and Headley SA. Carbohydrate-Protein Drink Improves Time to Exhaustion after Recovery from Endurance Exercise. JEPonline 4: 45-52, 2001.
7. Saunders M, Kane M, and Todd K. Effects of a Carbohydrate-Protein Beverage on Cycling Endurance and Muscle Damage. Med Sci Sports Exc 36: 1233-1238, 2004.
8. Saunders MJ, Luden ND, and Herrick JE. Consumption of an oral carbohydrate-protein gel improves cycling endurance and prevents postexercise muscle damage. J Strength Cond Res 21: 678-684, 2007.
9. Williams MB, Raven PB, Fogt DL, and Ivy JL. Effects of Recovery Beverages on Glycogen Restoration and Endurance Exercise Performance. Journal of Strength and Conditioning Research 17: 12-19, 2003.
10. Zawadzki KM, Yaspelkis BB, 3rd, and Ivy JL. Carbohydrate-protein complex increases the rate of muscle glycogen storage after exercise. J Appl Physiol 72: 1854-1859, 1992.

Chapter II: Review of the Literature

It is well known that carbohydrate is essential for recovery from prolonged, strenuous endurance exercise. However, recent research suggests that the addition of protein (PRO) to a carbohydrate (CHO) supplement can have substantial benefits beyond that of providing CHO alone. Several investigations have found that post-exercise CHO+PRO supplementation will in fact speed the recovery process and improve subsequent exercise performance (94, 173, 217, 257, 266). Therefore, in this review I will discuss the relevant literature regarding the effects of CHO+PRO supplementation on the following key areas of endurance exercise recovery and adaptation: glycogen resynthesis, immune system function and muscle tissue damage, initiation of protein synthesis, subsequent endurance exercise performance, and training adaptations (specifically, maximal oxidative capacity and maximal oxygen consumption).

ACUTE ENDURANCE EXERCISE PERFORMANCE

As mentioned previously, it is well established that endurance exercise performance is significantly improved when carbohydrate is ingested during exercise compared to water only or placebo beverages (37, 38, 99, 263, 264), yet recent investigations have reported significant improvements in endurance exercise performance when a carbohydrate-protein (CHO+PRO) beverage is ingested during exercise compared to a carbohydrate-only (CHO) beverage (100, 217, 219).

Ivy et al. (100) compared the effects of a placebo, CHO (7.75%) and CHO+PRO (7.75% CHO, 1.94% PRO) supplement on aerobic endurance performance. Nine trained cyclists exercised on 3 separate occasions at intensities that alternated between 45% and 75% of maximal aerobic consumption (VO_2max) for 3 hours, and then at 74% - 85% VO_2max until exhausted. Two hundred milliliters of randomly-assigned supplement was provided every 20 min during exercise. While CHO supplementation significantly increased time to exhaustion, the CHO+PRO supplementation extended time to exhaustion (TTE) by 36.5% (26.9 +/- 4.5 min vs 19.7 +/- 4.6 min, $p<.05$). Saunders et al. (217) reported similar TTE improvements with CHO+PRO compared to CHO

supplementation during exercise, and also found significantly reduced muscle damage in the CHO+PRO condition compared to CHO. Improving TTE is important to elite athletes, especially those undertaking prolonged events such as road cycling races or triathlons, but it is also attractive to recreational exercisers as well who seek to exercise longer and increase endurance capacity.

In addition to the need for exercise beverages to help improve endurance time, many athletes or recreational exercisers desire a lower caloric content alternative when maintaining or reducing body weight is a concern or goal. Since the addition of PRO to a CHO beverage has been demonstrated to improve the performance characteristics of a 6-8% CHO sports drink (100, 173, 217), it is likely that the CHO content of a sports drink could be substantially reduced yet benefit performance with the addition of a smaller amount of protein. In this manner, the total energy content of the beverage could be reduced up to 50%. Martinez-Lagunas et al. (140) recently compared the effects of a 4.5% CHO plus 1.15% PRO, and a 3% CHO plus 0.75% PRO beverage, to a traditional 6% CHO beverage and found that there was no difference in the times to exhaustion between the treatments. This suggests that the efficacy of the supplements was maintained despite the reduction in total CHO and total energy content with the substitution of a small amount of protein (140).

It is also known that the maximal rate of exogenous carbohydrate utilization is about 1.0-1.1 g/min when a single carbohydrate source (e.g., dextrose) is ingested (107-109, 251), but when multiple carbohydrate types (e. g., dextrose, fructose and maltodextrin) are ingested, this rate can be increased significantly (102, 104, 106, 107). Currell and colleagues (46) demonstrated an 8% improvement in time to complete a subsequent time trial when a mixture of glucose and fructose was ingested during a previous bout of prolonged exercise compared to glucose only. The use of different intestinal carbohydrate transporters for glucose and fructose is most likely the reason for the improved exogenous carbohydrate oxidation and thus, improved performance. Thus, it is possible that a lower-carbohydrate, moderate-protein and lower calorie beverage that utilizes different carbohydrates for maximal intestinal absorption could extend endurance

as well as, or better than, the traditional higher calorie alternatives.

While many investigations have demonstrated improved time to exhaustion with the addition of protein to a carbohydrate supplement ingested during exercise (100, 173, 217, 219), the mechanisms by which this improved TTE occurred has still not been clearly elucidated. One possible mechanism through which CHO+PRO supplementation improves performance is that CHO+PRO spares endogenous fuel supplies by decreasing muscle glycogen use and/or reducing the depletion of Krebs cycle intermediates. Cermak and colleagues (31) provided 6% CHO or 6% + 2% PRO to trained male subjects such that they received 60 g/h CHO +/- 20 g/h PRO during 90 min of cycling at ~69% VO_2peak. CHO+PRO ingestion increased the plasma branched chain and essential amino acids compared to CHO, but net muscle glycogen use and Kreb's cycle intermediate expansion was not significantly different between trials (31). The investigators concluded that when CHO is ingested at the higher end of the generally recommended exercise ingestion amount (60g/h), the addition of PRO does not improve substrate availability via Kreb's cycle imtermediates or spare muscle glycogen stores (31). However, key limitations in this study could have affected the results. For example, the only Kreb's cycle intermediates measured were citrate and malate, which are not rate-limiting intermediates. Also, the duration of the exercise bout was only 90 min and at a moderate intensity; this is not a difficult effort for trained subjects, and muscle glycogen would likely not be adequately depleted after this bout. Therefore, the findings of the study by Cermak and colleagues may be hindered by the study design.

Another proposed mechanism emerged from an investigation by Saunders and colleagues (217), in which 15 cyclists rode a cycle ergometer at 75% VO_2max to exhaustion, followed 12-15 hours later by a second ride to exhaustion at 85% VO_2max. Participants consumed 1.8 ml/kg body wt of a CHO or CHO+PRO beverage every 15 min of exercise and 10 ml/kg body wt immediately post-exercise. As occurred in the Ivy et al. (100) investigation, the cycling TTE was significantly increased in the CHO+PRO treatment. The cyclists rode 29% longer in the first and 40% longer in the second ride when consuming CHO+PRO compared to the CHO beverage. However, peak post-

exercise plasma creatine phosphokinase (CPK), an indirect measure of muscle damage, was also measured and was found to be 83% lower after the CHO+PRO trial compared the CHO trial (217). These findings suggest that the addition of protein to a carbohydrate supplement both increases aerobic endurance performance and reduces muscle damage caused by prolonged, intense exercise. In a follow-up investigation, Saunders and colleagues again examined the effects of CHO+PRO supplement on TTE and muscle damage as assessed by levels of creatine phosphokinase (CPK). TTE was extended by 13% with CHO+PRO, and CPK levels were significantly elevated in the post-exercise period in the CHO treatment, they did not rise in the CHO+PRO treatment, suggesting a significant reduction in muscle damage (219). It is possible that reducing muscle damage may be a mechanism through which the extended time to exhaustion occurs, although a few recent investigations demonstrated significant reductions in muscle damage without differences in TTE (210, 246).

Other recent investigations have substantiated the findings of improved TTE. Pratt et al. (199) had 10 non-elite triathletes perform two simulated duathlon trials consisting of an 8km run at 80% VO_2max, followed by a 50km ride at 70% VO_2max, and then a second run at 80% VO_2max (with no rest in between modes) to exhaustion. Subjects were provided either CHO or CHO+PRO during the cycling portion of the protocol. The team found that TTE was significantly improved in CHO+PRO versus CHO (mean = 4.5±7.1min; $p<.05$) (199). Likewise, Saunders et al. (219) reported increased TTE and decreased post-exercise muscle damage (CPK levels) when athletes consumed CHO+PRO gels versus gels containing CHO alone during cycling exercise.

Despite several reports of improved endurance performance with CHO+PRO, there are other investigations that have not corroborated this finding. Recent investigations using isocaloric CHO and CHO+PRO treatments found no difference in TTE (176, 210, 246). In addition, Van Essen and Gibala (247) found no difference in cycling time between isocarbohydrate CHO and CHO+PRO treatments when using a cycling time trial as the measure of performance.

It is likely that some of the discrepancies in the literature regarding the benefits or

lack thereof of adding PRO to a CHO supplement stem from differences in methodology. The method of defining and assessing endurance performance could be one such difference. Van Essen and Gibala (247) used time to complete and 80-km time trial as the measure of performance, finding no difference between a 6% CHO and a 6% CHO-2% PRO beverage. Studies by Ivy et al. (100) and Saunders et al. (217, 219) utilized TTE as the performance measure. Perhaps the benefit of PRO added to a CHO supplement is extending endurance time and delaying onset of exhaustion, which is important in ultra-distance events, triathlons, cycling stage races, and long cycling single-day events such as centuries (100 mi events).

The difference in ingestion rates used in the various investigations could also be a reason for conflicting findings. In the investigations by Ivy et al. (100) and Saunders et al. (217, 219), CHO ingestion rates averaged about 47 $g \cdot h^{-1}$, 37 $g \cdot h^{-1}$, and 41 $g \cdot h^{-1}$, respectively, whereas Van Essen and Gibala (247) used a rate of 60 $g \cdot h^{-1}$. Van Essen and Gibala (247) suggested that when CHO ingestion rates closely match the optimal rate of CHO oxidation, the addition of PRO to a CHO supplement coffers no additional performance benefits. While the maximal rate of exogenous carbohydrate utilization during prolonged exercise is about 1.0-1.1 $g \cdot min^{-1}$ (or about 60-70 $g \cdot h^{-1}$) when a single CHO source is provided (107, 108, 251), the combination of multiple carbohydrates ingested at a high rate (2.4 $g \cdot min^{-1}$), during exercise, the oxidation rate can increase to 1.75 $g \cdot min^{-1}$ (102, 103). It should be noted, however, that this is a large amount of CHO to ingest during exercise and may not be well tolerated by many exercisers. A more practical approach is to ingest a smaller caloric amount that maximizes both exogenous CHO oxidation and endogenous fat oxidation, and extends time to exhaustion.

Given that many of the studies examining the effects of CHO+PRO on endurance performance use relatively small sample sizes, Saunders et al. (218) examined data across multiple studies to determine if performance was in fact related to changes in physiological measures during exercise. To accomplish this, thirty eight subjects were combined from three studies in which cyclists performed rides to exhaustion at 75% VO2peak. In each study analyzed, cyclists received CHO (7.3%) or CHO+PRO

(7.3+1.8%) every 15 min during exercise, using a random, counterbalanced and double-blind design. VO_2, heart rate, blood glucose, blood lactate, RER and RPE were compared between the treatments at the 30-min time point during exercise. This time point was chosen because it was a common measurement time for all subjects in all of the studies examined. Despite finding no differences in VO_2 (2.89±0.58; 2.86±0.61 L/min), blood glucose (83.1±14.5; 82.4±17.8 mg/dL), and RER (.95±.03; .95±.03) between CHO and CHO+PRO treatments, TTE was 19% longer ($p<.05$) in CHO+PRO (116.5±33.9 min) than CHO (97.8±32.4 min). Heart rate (163.3±14.0; 159.3±14.1 bt/min), RPE (14.6±2.0; 14.1±2.0) and lactate (3.4±1.3; 2.9±1.5 mmol/L) were significantly lower ($p<.05$) during the CHO+PRO trials. The TTE differences were significantly correlated with treatment differences in exercise responses for heart rate ($r=.410$) and RPE ($r=.306$) but not lactate ($r=.065$) (218). Thus, the authors concluded that the combined data showed significant improvements in endurance performance with CHO+PRO versus CHO supplementation. While the decreases in HR and RPE were associated with improvements in performance, the magnitude of those differences did not appear large enough to explain the large improvements in performance with CHO+PRO treatments. The authors suggest that the underlying mechanism(s) underlying performance improvements with CHO+PRO beverages may also produce the physiological alterations in HR and RPE that were observed in this study (218).

In summary, it is reasonable to hypothesize that a lower-carbohydrate, moderate-protein supplement containing fewer calories relative to a higher carbohydrate and protein beverage can extend time to exhaustion during prolonged endurance exercise, and potentially reduce muscle damage as well.

MUSCLE GLYCOGEN RESYNTHESIS

The Importance of Muscle Glycogen in Recovery from Endurance Exercise

The importance of muscle glycogen as a fuel source during prolonged strenuous exercise is well established. It has been demonstrated that aerobic endurance is directly related to the initial muscle glycogen stores (14, 15). Once these stores are depleted, strenuous exercise cannot continue, and even the perception of fatigue rises as muscle

glycogen levels fall (1, 14, 15, 78). Thus, the replenishment of muscle glycogen is of great importance for adequate recovery after intense training or competition and has a substantial impact on physiological readiness for subsequent exercise bouts.

It is important to note that how investigators define moderate exercise or intense exercise can vary from study to study. Generally, and for purposes of this review, moderate endurance exercise is defined as <60 min and <65% VO_2max, while prolonged, strenuous exercise is defined as >90 min and >65% VO_2max.

Amount and Timing of Post-Exercise Carbohydrate Supplementation and Glycogen Synthesis

With sufficient CHO intake post-exercise, restoration of muscle glycogen to baseline levels can occur in 24 h (15, 28). However, for athletes who must train or compete again without the luxury of adequate restorative time, a more strategic approach must be employed in order to optimize glycogen replenishment to fuel the next exercise bout. Much research has been performed to elucidate the optimal amount and timing of post-exercise CHO supplementation for muscle glycogen resynthesis. Essentially, at least 0.7g CHO/kg body weight/h is needed immediately and 2-hrs post-exercise to achieve a muscle glycogen restoration rate of approximately 7 mmol/kg/wet wt/h (19, 96, 98). However, this rate of resynthesis is apparently not the maximum that can be achieved, as has been demonstrated by investigations using greater supplementation frequencies (e.g., every 15 or 30 min) (48, 196, 248). In these instances, the CHO provided was at a rate of ~1.0 to 1.2 g CHO/kg/hr, and suggests that muscle glycogen storage can be up to 30% more under these conditions than when CHO is provided at 2 h intervals. However, on a practical level, consuming this much CHO at 15 to 30 min intervals post-intense exercise is often unrealistic and undesirable, as this is a large amount to consume for most athletes. Therefore, a more practical and beneficial approach for maximizing muscle glycogen replenishment is preferred, and it appears that this can be achieved by adding protein to a CHO supplement.

Effects of Carbohydrate plus Protein on Post-Exercise Muscle Glycogen Resynthesis

It is known that muscle is more sensitive to insulin following exercise (95) and that providing a CHO supplement immediately post-exercise results in a rate of glycogen synthesis twice as fast as providing the same supplement 2-hrs post-exercise (96). Investigations have shown that CHO intake greater than 0.75 g/kg/h body wt will maximize muscle glycogen storage when provided at 2-h intervals (98) and that the rate limiting step in muscle glycogen resynthesis is evidently not glucose delivery to the muscle (206). Combining PRO with CHO supplementation was found to significantly increase the post-exercise insulin response to CHO and accelerate the rate of muscle glycogen resynthesis beyond that of an isocarbohydrate (266) and isocaloric CHO supplement (94). Taken together, these findings suggest that the addition of PRO to a CHO supplement appears to increase the rate and efficiency of muscle glycogen synthesis.

The study performed by Zawadski et al. (266) was the first to investigate the combined effect of CHO+PRO on post-exercise muscle glycogen synthesis. Nine subjects performed 2 h of glycogen-depleting cycling exercise and then were provided one of three treatments immediately and 2 h post-exercise: 112 g CHO, 112 g CHO + 40.7 g PRO (total of 152.7 g), or 40.7 g PRO. Biopsies were taken immediately after exercise and 4 h later. Relative to the CHO treatment, the CHO+PRO treatment resulted in a 38% greater rate of muscle glycogen resynthesis across the 4 hours of recovery. Little glycogen storage occurred with the PRO treatment. Insulin levels were significantly greater with CHO+PRO, and the investigators attributed the faster rate of glycogen synthesis (35 versus 25 μmol/g muscle protein) to the increased insulin response (266). However, the findings met with much controversy given that the treatments were not isocaloric (difference of 162.8 kcals per dose), despite previous studies that demonstrate that the addition of calories when the total caloric content is adequate does not achieve a greater rate of glycogen resynthesis and storage (98).

Investigations by other laboratories (26, 105, 236, 248, 250) failed to confirm the findings of Zawadski et al. (266). However, rather than finding a negative effect of

CHO+PRO versus CHO alone, these studies simply found no significance difference between the two in terms of glycogen resynthesis. But it is important to note that there are obvious differences in the methodologies of these studies that may account for the difference in conclusions. Differences in both the timing/frequency of supplementation, as well as in the amount of macronutrients and total energy provided during the recovery period likely account for much of the differing reports.

For example, Tarnopolsky et al. (236) used a very small amount of protein – 0.1 g/kg -- in addition to 0.75 g CHO/kg body wt to compare against 1 g/kg CHO or placebo and found no significant differences between the CHO and the CHO+PRO supplements, although both increased glycogen resynthesis significantly above placebo. However, given the small amount of protein added to the supplement, and given that the CHO+PRO combination resulted in less total kcals than the 1 g/kg CHO supplement, it is not difficult to understand why this would fail to demonstrate an advantage. Other investigators provided a greater amount of CHO (1.2 g/kg/h divided into doses every 30 min for 3 h) along with CHO+PRO and found that while the CHO+PRO did increase insulin levels, there was no significant difference in muscle glycogen synthesis, suggesting that as long as the amount of CHO provided is high enough, there is no benefit to be gained from the addition of protein (105). Compared to the studies described above, Van Hall et al. (248) provided more CHO (1.67 g/kg body wt and 1.67 g CHO plus 0.5 g/kg protein) at a greater frequency (every 15 min for 4 h). They found that the addition of PRO did not increase leg glucose uptake or glycogen resynthesis, and concluded that high amounts of CHO alone is enough to maximize resynthesis. However, these amounts of CHO should be considered excessive, and is not practical as a post-training and competition strategy.

Carrithers et al. (26) also used less PRO (only 0.2 g/kg/h PRO with 0.7 g/kg/h CHO) and more frequent supplementation intervals (every 30 min for 4 h), and likewise failed to find a significant difference in muscle glycogen resynthesis. However, the differences as described above from the Zawadski et al. (266) protocol could certainly account for what seems to be a null hypothesis--that adding PRO to CHO does not

improve muscle glycogen resynthesis beyond that achievable by adequate CHO alone. Recently, Howarth and colleagues (89) assessed muscle glycogen resynthesis when subjects were provided CHO and CHO+PRO treatments following 2 h of cycling exercise. The three randomly-ordered treatments provided either 1.2 g/kg/h CHO, 1.2 g/kg/h CHO + 0.4 g PRO, or 1.6 g/kg/h CHO. Muscle biopsies were taken from the vastus lateralis to determine the muscle glycogen resynthesis that occurred over a 4-h recovery period. The glycogen resynthesis rate was not significantly different between the 3 treatments, nor were the plasma glucose and insulin response. These results suggests that a 1.2 g/kg/h CHO ingestion rate is sufficient to optimize muscle glycogen storage, and that adding 0.4 g/kg/h of either protein or additional carbohydrate does not further increase this process (89).

Of these inter-study differences in supplementation frequency, CHO+PRO amounts and total energy content, a few important points should be addressed. Ivy et al. (98) showed that merely adding calories (adding more CHO to a CHO supplement) is not sufficient to continue increasing the rate of muscle glycogen resynthesis. Providing 3g CHO/kg/h versus 1.5 g CHO/kg/h had the same effect on muscle glycogen resynthesis. Therefore, simply adding energy when the energy amount in the supplement is already sufficient for muscle glycogen restoration is not an issue. Increasing the feeding frequency may not show a clear benefit from the addition of PRO to the CHO supplement, but are large boluses every 15 or 30 min really practical in real-world settings? More often than not, athletes consume a recovery supplement immediately after exercise, and then 1-2 h later. A strategy that addresses the practicality of daily training or competitive events, and that maximizes muscle glycogen restoration, is much more attractive and useful.

Finally, another key difference in study design can also account for varied findings: different methods of assessing muscle glycogen levels. The gold standard has long been the needle biopsy, and it is performed successfully and reliably in laboratories all over the world. However, with the emergence of NMR (nuclear magnetic resonance) technology, assessments can be made non-invasively and at much more frequent intervals

than with the traditional biopsy method. Thus, more measurements are able to show a more detailed timecourse and sample a greater area of the thigh for more accurate measurements. Two studies that did in fact show significantly greater muscle glycogen resynthesis in response to CHO+PRO versus CHO alone used this method (13, 94). Ivy et al. (94) found a significant increase in glycogen resynthesis during recovery at 40 min and at 4 h (88 mmol/L with CHO+PRO versus 75.5 mmol/L with an isocaloric CHO supplement, versus 70 mmol/L with an isocarbohydrate supplement). While there was not a significant difference in insulin responses between the three treatments, blood glucose was lower in recovery with CHO+PRO, suggesting that more glucose was being converted to glycogen and stored. Interestingly, at the end of the 4 h recovery, 46% of the glycogen used was restored in the CHO+PRO treatment, versus 31% and 28% restored in the other 2 treatments (isocaloric and isocarbohydrate, respectively) (94). Berardi et al. (13) reported similar results using isocaloric treatments (CHO+PRO contained 0.8 g/kg CHO and 0.4 g/kg PRO and CHO contained 1.2 g/kg CHO) given immediately and at 1 and 2 h post-exercise. Greater glycogen resynthesis was found after a 6 h recovery period in response to the CHO+PRO treatments (13).

Recently, our laboratory investigated the effects of cereal with nonfat milk (Cereal) versus a traditional sports drink (Drink) immediately following prolonged aerobic exercise on muscle glycogen synthesis and the activity of enzymes controlling glycogen storage and mRNA translation (i.e., glycogen synthase, Akt, mTOR, rpS6K and eIF4E) (110). While both treatments raised plasma glucose and insulin levels during recovery, interestingly, Cereal raised plasma insulin significantly higher and blunted the rise in blood lactate relative to Drink. Although muscle glycogen storage was similar between the two treatments, mTOR phosphorylation was greater following recovery with Cereal verses Drink. As will be discussed later, mTOR is a key protein regulating not only glycogen synthesis, but importantly, for regulation of protein synthesis. In addition, only Cereal significantly altered the phosphorylation status of glycogen synthase and Akt during recovery indicating greater activation. Thus, Cereal had significant effects on the enzymes that are essential to the pathways for glycogen and protein synthesis, increased

insulin levels that appeared to facilitate muscle glucose uptake and glycogen storage, and blunted lactate formation during recovery, which suggests that more intracellular glucose was indeed converted to glycogen. Glycogen synthase also appeared to remain more active after Cereal compared to Drink despite higher glycogen levels (110).

The findings of the many studies discussed above indicate that combined ingestion of CHO+PRO as a recovery supplement can indeed increase the rate and amount of muscle glycogen resynthesis when taken immediately post-exercise and at frequencies of 1-2 h. CHO+PRO supplementation may be especially beneficial when the amount of CHO that would otherwise be ingested is less than optimal (i.e., less than 0.8 g/kg body wt). This is particularly important when an athlete has only a few hours between training sessions or competitive events.

As will be discussed in detail below, there are many other benefits to the athlete of adding PRO to recovery supplements that extend well beyond simply optimizing glycogen stores, which is a worthy goal in and of itself. These benefits include protecting immune system function, reducing muscle damage, optimizing mRNA translation initiation for increased protein synthesis, improving performance in subsequent exercise bouts, and increasing the rate of training adaptations.

IMMUNE CELLS, INFLAMMATORY MARKERS, AND MUSCLE DAMAGE

The immune response to prolonged, intense endurance exercise has received much attention in recent years, and the specialized area of exercise immunology has become a relatively new and exciting niche field of research. Exercise immunology is a very broad area and encompasses all aspects of the immune system, as well as interactions with multiple systems such as the endocrine and nervous systems and their dynamic interplay with prolonged and strenuous exercise. Therefore, in order to fully understand the effects of training and supplementation down to the cellular level, the immune response to intense exercise must be more clearly elucidated.

Over the past 25 years, many studies have shown that exercise induces changes in immune system function. These changes differ, however, based on the intensity and duration of the exercise. It is well accepted that regular moderate exercise is associated

with reduced incidence of bacterial infection compared with a completely sedentary state (69). In contrast, prolonged, strenuous bouts cause a temporary suppression of the immune system that lasts for several hours after exercise, and this immune dysfunction is most pronounced when the exercise is continuous, prolonged (>1.5 h), of moderate to high intensity (55 - >75% VO_2max), and performed without food intake (69).

It is well established that intense exercise can negatively impact the immune system, resulting in decreased immune function and higher rates of illnesses such as upper respiratory tract infections (70, 154, 156, 158, 167). Many nutritional countermeasures have been investigated for potential impact on immune variables in response to endurance exercise, including CHO beverages, glutamine, bovine colostrum, phytonutrients such as quercetin, and antioxidants such as vitamins C and E (159, 162, 164, 165, 224). Of these, carbohydrate ingestion has been demonstrated to be by far the most effective against many immune and inflammatory outcome measures (154, 158, 159). The effects of CHO+PRO on immune response to exercise in such a model as this have never before been investigated, and thus much of the background for this review will draw from investigations using CHO supplements post-exercise. The effects of strenuous and prolonged endurance exercise alone will be reviewed in brief in order to demonstrate the potential importance of providing CHO+PRO after exercise to fully protect immune function.

For the purposes of this review, I will focus on a few key immune parameters in relation to CHO+PRO supplementation post-endurance exercise: (1) two subpopulations of leukocytes -- monocytes and neutrophils; and (2) five key cytokines known to be impacted by strenuous exercise – IL-1Ra, IL-6, IL-8, IL-10, and TNFα. Additionally, it is impossible to discuss the effects of exercise on the immune system without concurrently discussing muscle damage, as the two are intimately linked. Therefore, this discussion will also include two blood markers of muscle damage: creatine kinase (CK; also known as CPK, creatine phosphokinase) and myoglobin.

Lymphocytes, Monocytes, and Neutrophils

The link between prolonged, strenuous endurance exercise and decreased immune function, as well as the immune-bolstering effect of moderate exercise, has been established and well reviewed (154, 189). For several hours after prolonged, intense endurance exercise, the number of circulating lymphocytes in the blood remains decreased, and immunity is impaired (154, 186). This period of immunosupression and increased risk of pathogenic attack is often called the "open window," and may last from 3 to 72 hours (172, 187). However, not all leukocytes respond in the same fashion. Prolonged, intense endurance exercise is associated with a biphasic perturbation of circulating leukocytes (66, 74, 168). Total leukocytes increase as much as 100% immediately post-exercise, with this increase evenly distributed by lymphocytes and neutrophils (NPs), and a smaller contribution made by monocytes (153). After longer events such as marathons, the increase has been shown to be much larger (200 – 300%) (157). However, within 30 minutes after exercise, total lymphocyte levels actually fall to 30% - 50% below pre-exercise levels and remain low for 3 – 6 h (168). The increase in lymphocyte concentration in the circulation immediately post-exercise is likely due to the release of all subpopulations into the blood during exercise (185), when heart rate and stroke volume - and thus, cardiac output - is increased to the active skeletal muscles and the cardiopulmonary system.

Contrary to the initial rise and then fall in lymphocyte concentrations post-exercise, however, NPs have been found to continually increase (neutrophilia) post-exercise (142, 168, 185). An elevation in circulating NPs was also found after only 30 min of brisk walking exercise (161). In response to 45 min of running at 80% of VO_2max, NP numbers were found to be increased from pre-exercise levels for ~8 h post-exercise (168). This increase could translate to a heightened immunosurveillance against any possible invaders such as upper respiratory tract infection (URTI) causing pathogens. However, the increase may not necessarily mean a heightened immune effectiveness but rather an increased presence of immature, less battle-ready NPs. Following intense or prolonged endurance exercise, there is an increased release of younger, less mature NPs called band neutrophils into the circulation (234). This is often called the leftward shift.

Mature NPs are normally the most abundant leukocyte in the blood and are the first leukocytes to respond to bacterial infection. They are powerful pathogen killers due to their ability to engulf pathogens and destroy them via oxidative burst. NPs are non-dividing cells, and have only a 1–4 day lifespan. If more immature NPs are circulating during the open window period, this could mean a less powerful response to a pathogen.

The decrease in total lymphocytes and the increased neutrophilia post-exercise is likely due to the increase in stress hormones that are released in response to prolonged exercise. Cortisol, the catecholamines epinephrine and norepinephrine, and growth hormone have been shown to modulate NP function (82, 231, 233). These three types of stress hormones are reported to exert differing effects, as detailed in a review by Pedersen et al. (184). The catecholamines, especially epinephrine, appear to be responsible for the immediate effects of exercise on lymphocyte subpopulations and cytotoxic activities, and catecholamines and growth hormone mediate acute effects of exercise on NPs, whereas cortisol is most likely responsible for maintaining the lymphopenia and neutrophilia seen after prolonged exercise (184). Cortisol, unlike the catecholamines, exerts its effect after a lag time of hours after exercise, so it is not likely to have an acute effect during or immediately after a prolonged bout (185). In addition, cortisol does not increase significantly in brief exercise but rather comes into play in prolonged bouts, and therefore is likely to contribute to the neutrophilia seen after prolonged exercise (65). However, given that catecholamines have been shown to inhibit NP activity (152), and that NPs may be important effectors of exercise-induced muscle damage that occurs during exercise (234), it is important to blunt the increase in circulating catecholamines during the prolonged exercise so that NP function during the bout is not impaired. In addition, minimizing the detrimental effects of cortisol during recovery is of great importance for bolstered immune function during the open window period.

While NPs are first on the scene of infection or injury, monocytes are also part of the primary leukocyte defense against a pathogenic attack. Monocytes (MOs) are unique in that they react to inflammatory signals of an infection and move to the site of insult within hours, where they differentiate into powerful phagocytic macrophages and

dendritic cells. Similarly to the NP response, MOs have been shown to increase during exercise and remain elevated afterwards (169, 208). MOs in the early differentiation stage are strongly recruited into the circulation during long-term aerobic exercise, whereas mature monocytes (premacrophages) increase most with high intensity anaerobic exercise (65). Interestingly, whereas bacterial infection induces the actual maturation of immature monocytes, exercise does not, which demonstrates the possible difference between change in actual cell counts or concentration and change in the actual functional capacities of those cells (65). Arnold et al. (6) recently reported that injured skeletal muscle recruits MOs that quickly differentiate into anti-inflammatory macrophages that then stimulate myogenesis and fiber growth. Viewed in this light, MO are potentially useful in supporting myogenesis and fiber growth. Clearly, MOs are an important part of the immune response to exercise and may mediate more than simply host defense in the post-exercise period.

Ingesting CHO during prolonged exercise has repeatedly been found to attenuate the typical increase in plasma stress hormones, such as cortisol, while lowering blood neutrophil and monocyte counts (155, 159, 166, 169, 171). Surprisingly, the potential for added benefits to be gained from adding protein to an exercise or recovery drink has not yet been investigated. Therefore, given that CHO supplementation alone significantly reduces some aspects of the decline in immune function in response to intense exercise stress, and given that CHO+PRO has many demonstrated beneficial effects on performance and recovery that supersede the effects of CHO alone, including reductions in muscle damage (11, 217), it is reasonable to hypothesize that CHO+PRO will improve immune function in response to intense exercise to a greater extent than CHO alone or placebo.

Cytokines

Cytokines are potent cell-to-cell signaling proteins that regulate inflammation and immune responses. While they can exhibit hormone-like actions, unlike hormones, they are made on demand rather than stored, are effective in very small concentrations, usually

exert their effects in a local range, and are virtually absent in healthy, stress-free subjects. However, cytokine levels increase significantly, even dramatically, during disease, tissue injury, and repair. Their pleiotropic actions are not limited to immune response only, as cytokines target multiple cell types and exert a wide range of effects.

Much of what is known about cytokines comes from sepsis research, which has revealed that the cytokine cascade is comprised of TNFα, IL-1α, IL-6, IL-1Ra, and IL-10, in that order (3, 192). Cytokines are produced and released at the site of inflammation as part of the local response to infection or tissue injury. They mediate the influx of many types of immune cells, including neutrophils and monocytes, to the area to clear away antigens, debris, and facilitate healing and repair (178). The local response combined with the systemic response that follows is called the acute phase response and is led by TNFα, IL-1α, and IL-6. The cytokine response to exercise differs somewhat from that brought about by severe infection, as indicated by a lack of large, significant increase in the pro-inflammatory cytokines TNFα and IL-1B with strenuous exercise (192), although some studies have reported small but statistically significant TNFα increases (50, 55, 178). IL-6, however, appears to be a key, possibly main, mediator of the acute phase response, and its elevation is used as an indicator of inflammation because it precedes the increase in other acute phase proteins (154). In addition to being the first cytokine to appear in response to intense exercise, Interleukin-6 (IL-6) also demonstrates the largest measurable increase--up to 100-fold during exercise before declining in the post-exercise period (59, 185, 188, 192, 232). At present, it appears that the exercise-induced cytokine cascade involves an increase in IL-6, followed by significant increases in the anti-inflammatory cytokines IL-1Ra and IL-10 (185).

IL-6 acts as both a pro and anti-inflammatory cytokine and is secreted by T and B cells, monocytes and macrophages to stimulate immune response to trauma or injury. Thus, it is often termed inflammation responsive rather than pro- or anti-inflammatory (178). In addition, IL-6 is released from skeletal muscle during contraction and its increase in response to exercise has been well documented (150, 177-179, 229, 245). A

versatile cytokine, IL-6 has been implicated as also having important roles in influencing glucose homeostasis during exercise (58) and in modulating fat metabolism (193, 249).

Much of the current IL-6 and exercise research has been performed by Pedersen and colleagues at the Copenhagen Muscle Research Centre. In one investigation, they studied trained male marathoners to determine the extent and time course of cytokine response to the strenuous exercise. They found that strenuous, prolonged running elicited a dramatic increase in IL-6, as well as an increase in the pro-inflammatory cytokines TNFα, IL-1B, two cytokines that often show no detectable increase from an exercise stimulus (177-179). They also found that this increase in pro-inflammatory cytokines was balanced by corresponding increases in cytokine inhibitors IL-1Ra and soluble TNFα receptors, as well as the anti-inflammatory cytokine IL-10 (178). IL-10 inhibits the release of TNFα and IL-1B (32), which may explain why these two classic pro-inflammatory cytokines often do not show a large significant increase in exercise. IL-10 also induces production of IL-1Ra (29, 101), which is the antagonist of the IL-1 receptor, again possibly explaining a lack of increase in IL-1B with exercise.

While many investigations have reported cytokine release in response to muscle damaging eccentric exercise, some have questioned whether cytokines are released in significant amounts in response to concentric or less muscle fiber-damaging exercise such as cycling. However, many studies of cycling have indeed reported significant cytokine elevations and immune perturbations post-exercise (122, 144, 163, 169, 171, 220, 221, 265). Also, Sprenger et al. (229) studied runners that completed a moderate-intensity run that caused very little muscle damage as assessed by a low increase in creatine kinase. Despite very little evident muscle damage, levels of TNFα and IL-6 were elevated, indicating that cytokine release occurs in response to many types of prolonged, strenuous exercise that is sufficient to generate a stress hormonal response and immune perturbation (229). Since damage to skeletal muscle can occur from the catabolic hormonal milieu (elevations in stress hormones such as the catecholamines and cortisol) that occurs after such exercise, even without a purely eccentric component, it is reasonable to expect an increase in cytokines in response to prolonged, strenuous

endurance exercise such as cycling. The findings of Sprenger et al. (229) support this concept.

As mentioned previously, some studies have demonstrated a significant increase in plasma TNFα concentrations in response to exercise (50, 55, 178, 229), but many others have failed to show changes in this cytokine (177, 179, 245). TNFα is of interest because of its role as a cytokine involved in systemic inflammation. In addition to inducing inflammation, TNFα induces many other powerful biological events, including apoptosis, cell proliferation, differentiation, and tumor growth, although regulation of immune cells appears to be its primary function. Overproduction of this cytokine has been implicated in cancer and many other pathologies. Whether or not it increases significantly in exercise is still unclear, probably due to methodological and protocol differences, as well as assay sensitivity issues. It is possible that there is a greater role for this cytokine in the exercise response, much like the response seen in trauma patients. Therefore, it seems clear that TNFα should be investigated further given its powerful effects on the human body. Differentiations in its response to exercise of various intensities and durations should be better elucidated in order to fully understand the effects of exercise on the inflammatory cascade and the immune system.

While much attention is paid to elevations in IL-6, IL-10 and IL-1Ra, as well as the potential increases in TNFα, the IL-8 response to prolonged, strenuous exercise is less characterized in the literature. This chemokine is secreted by several cell types including macrophages and epithelial cells, and is also expressed in working skeletal muscle. IL-8 has important functions as a chemoattractant and potent stimulator of angiogenesis. The dearth of information on its response to, and role in, exercise may be due to the difficulty of detecting it in plasma, as it has small, transient increases that act locally rather than via traveling through the circulation (183). Recently however, a few studies have demonstrated significant increases in this chemokine in response to cycling exercise (2, 64), as well as knee extensor exercise (2).

Akerstrom et al. (2) aimed to find if IL-8 is released from exercising muscle fibers. They had seventeen healthy subjects perform two independent trials: cycling at

moderate intensity for 3 h, and also performing 3 h of two-legged knee-extensor exercise at 60% of maximal workload. Muscle biopsies of the vastus lateralis were taken in each experiment. The investigators reported a significant increase in IL-8 mRNA after exercise, and a marked IL-8 expression in biopsy samples obtained during the recovery phase following 3 h of cycling. The peak actually occurred 3-6 h post-exercise. In the knee extensor protocol, a small transient net release of IL-8 was found at 1.5 h during exercise. This small release did not result in an increased plasma concentration of IL-8. The investigators concluded that muscle-derived IL-8 may indeed play a key local role in muscle fibers, such as stimulating angiogenesis (2). More recently, Frydelund-Larsen et al. (64) examined the effect of cycling exercise at moderate intensity on IL-8 mRNA and protein expression within vastus lateralis fibers. Because IL-8 has been shown to stimulate angiogenesis by binding to the CXC receptor 2 (CXCR2), they measured CXCR2 mRNA and CXCR2 protein as well. The team had healthy volunteers cycle for 3 h at 60% VO_2max. Muscle biopsies were taken from the vastus lateralis before exercise, immediately post-exercise, and at 4.5, 6, 9 and 24 hours post-exercise. CXCR2 mRNA increased post-exercise, and the protein had increased at 4.5 hours. Taking the findings of their earlier study (2) a few steps further, the investigators concluded that concentric cycling exercise induces CXCR2 mRNA and protein expression in the vascular endothelial cells of the muscle fibers, and reasoned that IL-8 likely acts locally to stimulate angiogenesis through CXCR2 receptor signaling (64). Given that angiogenesis is an important training adaptation that serves to increase the transport of nutrients and oxygen to working muscle, as well as facilitate the speedy removal of metabolic waste from the fibers, IL-8 is worthy of more attention in future investigations.

There are several notable reasons for the discrepancies in the findings of cytokine responses to exercise. One such issue is simply detecting them in the blood. High sensitivity assays must be used, and even then the changes can be transient, as some of the cytokines disappear rapidly after exercise. According to the work of Ostrowski et al., (177-179) IL-6 peaks immediately after exercise, while IL-1Ra peaks 1-2 h post-exercise, and TNFα may show a slight increase immediately post-exercise. Therefore, when

measuring cytokine levels in response to exercise it is critical to sample at the correct times. Also, most cytokine increases have been detected after eccentric exercise, since this type of exercise is more muscle damaging than concentric. However, large and significance increases are in fact found in response to endurance exercise of a concentric nature (i.e., cycling). However, variations in the intensity and duration of exercise protocols can contribute to conflicting findings. In addition, specificity and cross-reactivity can be a problem with detecting a cytokine of interest, as it is possible that more than one cytokine can be mistakenly measured, leading to inflated concentration values. With careful biochemical methodologies and skills employed in a well-designed and executed protocol, these difficulties can be overcome in detecting cytokines in response to prolonged, intense endurance exercise.

It seems reasonable to hypothesize that when the immune system is functioning at a normal, healthy level, a system of inhibitors helps to keep the inflammatory process in response to strenuous exercise in check. How to encourage this process so the balance between allowing the right amount of post-exercise inflammation that is useful for clearing tissue damage and stimulating adaptation, and producing too great an inflammatory, immunosuppressive effect, is achieved is an area for further research and elucidation. At present, nutritional supplementation with carbohydrate has been well studied and is the most effective way to achieve an attenuation in the cytokine and inflammatory response to exercise.

It is known that ingesting CHO compared to placebo during prolonged endurance cycling or running attenuates the significant increase in cytokines such as IL-6, IL-8, IL-10 and IL-lra (150, 158, 159, 163, 166). CHO ingestion compared with placebo also has been demonstrated to attenuate exercise-induced changes in plasma cortisol and epinephrine. In a study of trained marathon runners, this attenuation was demonstrated, and as would be expected, blood glucose levels were higher in the CHO group, which was negatively correlated with cortisol levels (160). This decrease in cortisol levels in response to CHO ingestion was also correlated with decreased leukocyte trafficking (160). Nehlsen-Cannarella et al. (150) also reported attenuated cytokine release after 2.5

h of intense running at 75% of VO_2max when a 6% CHO beverage was provided instead of placebo only. Clearly, running has an eccentric component to the exercise, but similar results have been shown in cycling. Recently, Scharhag et al. (220) studied the administration of 6% or 12% CHO beverages given during 4 h of cycling in trained subjects, and reported that the ingestion of at least 6% CHO beverages can sufficiently attenuate the exercise-induced immune response and stress, especially the phagocytic neutrophils and monocytes via a reduction in cortisol release. Interestingly, increasing the concentration to 12% CHO conferred no advantage or improvement in immune function over that provided by the 6% beverage, except for an attenuation in CRP (Scharhag et al., 220).

Taken together, these results strongly indicate that nutritional supplementation during exercise can have a significant positive effect in attenuating the inflammatory cascade and immunosupression following intense and prolonged exercise. To our knowledge, the combination of CHO and PRO ingested during exercise rather than simply CHO alone has not been investigated. It seems reasonable to hypothesize that the addition of protein could in fact bolster the immunobenefits of CHO ingestion by reducing or attenuating the catabolism of muscle protein during and after intense endurance exercise to provide substrate for gluconeogenesis, as well as by providing fuel for the immune cells themselves (the amino acid glutamine is used as a primary fuel source for immune cells). In addition, the addition of protein has been shown to speed the recovery process from intense endurance exercise, as previously discussed, and enhancing the recovery process inherently should translate to less immune stress and perturbation in the critical post-exercise period.

Immune function in trained versus untrained populations

While much is known about the effects of endurance exercise and training on immune function, less is understood about potential differences, if any, in the acute immune response to exercise between untrained and trained individuals. Both animal and human studies have been performed to assess differences in these training states, but have

reached disparate conclusions. In a study with trained versus untrained rats, the untrained animals demonstrated a significantly greater suppression of immunocompetence after exhaustive exercise than the trained rats, which only showed a slight suppression (136). In this study, immunocompetence was assessed by measuring the blastogenic response to concanavalin A by spleen cells (136). Other investigations have used lymphocyte proliferation and subpopulations as an indicator of immune function. For example, Lin et al. (131) examined the effect of an acute bout of exercise at 70% VO_2max in trained and sedentary rats and found that the mitogenic activity of spleen lymphocytes in the trained rats to staphylococcal enterotoxin B, which activates T cells, decreased compared to the sedentary group after the run. However, the lymphocyte proliferative response to lipopolysaccharide, a B cell mitogen, was bolstered (131). Therefore, while there was a tendency for some immune cell populations to increase or decrease activity in the trained versus untrained groups, no statistically significant differences were found overall (131).

MacNeil et al. (135) investigated the effects of cycling exercise intensity and duration on lymphocyte proliferation in men of three fitness levels: sedentary to low (VO_2max = ~44.9 ml/kg/min), moderate and recreationally active (VO_2max = ~55.2 ml/kg/min), and high and endurance trained (VO_2max = ~63.3 ml/kg/min endurance trained), in addition to a mixed control group (VO_2max = ~52.4 ml/kg/min). Subjects completed four cycling bouts ranging in duration from 30 – 120 min and in intensity from 30% - 65% VO_2max. Blood samples were obtained at various times before and after the exercise sessions. The investigators reported depressed mitogenesis in all groups 2 h after the exercise bouts, but found that the reduction in lymphocyte proliferation to the concanavalin A mitogen after exercise was transient, with recovery to pre-exercise levels within 24 h post-exercise (135). These findings suggest that acute bouts of submaximal exercise transiently reduce lymphocyte function in men and irrespective of the individual fitness level. It should be noted, however, that the VO_2max values for the participants were higher than would normally be classified as low and moderate, and the intensity levels of the rides were low in a population with VO_2max values of 44 ml/kg/min and above.

Using natural killer (NK) cell activity as a marker of immune function, Pedersen et al. (190) demonstrated increased NK cell function in highly trained cyclists compared to untrained controls, which may result in better resistance against infection in trained people versus the untrained. Recently, Selkirk et al. (223) examined endotoxin leakage and inflammatory activation during exertional heat stress in trained and untrained humans. Subjects walked at 4.5 km/h with 2% elevation in a climatic chamber heated to 40^{o} C until exhaustion. The team found that while heat stress increases the concentration of circulating endotoxin and cytokines in both groups, this response occurs at a lower temperature in the untrained than in the trained, suggesting a greater immune tolerance to heat exertion in trained people (223).

It is important to note that none of the above studies of differences in training status and immune function have used nutritional supplementation as a possible way to mediate this response. This is likely due, in part, to the lack of consensus on what the difference in the trained versus untrained groups may be. As explained in a review on exercise and the immune system by Pedersen and Hoffman-Goetz (185), lymphocyte proliferative responses have been shown in many studies to increase, decrease or not even change when comparing athletes and the untrained, and the same applies to NP function. Clearly, this is an area for more research to be performed in order to understand any potential differences in training status, and how best to mediate them.

Immune function in periods of intense training

In a review of immune function in athletes, Mackinnon et al. (134) reported that extended periods of intense training are associated with decreases in immune parameters such as neutrophil function, suggesting possible immune suppression in trained athletes. In fact, it has been suggested that periods of intensified training (overreaching) lasting 1 week or more may result in more prolonged immune dysfunction than what is observed after an acute bout (69). However, not all studies in trained athletes have reported such suppression (185, 231). Suzuki et al. (231) reported that after 1 week of intense endurance training by healthy, untrained men, the only parameter to show a decline was

resting neutrophil count. One obvious problem with extrapolating this finding to the effects of chronic training is the short duration used by Suzuki et al. (231), since most endurance athletes train for far greater periods, such as years. Decreases in total circulating leukocytes in response to chronic endurance training have been reported (18, 120). It is possible that the well-documented plasma volume expansion seen in trained endurance athletes (40, 42, 88) is responsible for decreases observed in total leukocyte concentration, since with increased blood/plasma volume, the population of leukocytes as a percentage of the blood will decrease. Even in the case of endurance training for one week, plasma volume increases in response to the training sessions (10) and could conceivably account for the decline in resting NP levels reported by Suzuki et al. (231).

Despite some evidence that immune function is suppressed in long-term training athletes, there is also evidence that a decrease in total circulating leukocytes and NPs is transient, with a training adaptation occurring in response to daily training (231, 234, 242). As noted by Neiman in a review on exercise and immunity, most studies have failed to show major training effects on circulating total leukocyte concentrations and their subpopulations, and many studies of resting serum immunoglobulin levels have not shown a significant difference in these levels between trained and untrained subjects (153).

The cytokine response to long-term endurance training is currently not very well characterized. Smith (225) proposed a cytokine theory of overtraining, in which exercise-induced damage and stress to the musculoskeletal system induces a local inflammatory response, which causes overtraining symptoms to develop. Over time, as the athlete fails to recover fully and properly yet continues to train, the inflammation is compounded and becomes chronic. Systemic inflammation then develops as pro-inflammatory cytokines are released from activated monocytes, and sickness behavior results (225). Parry-Billings et al. (180) had previously investigated overtraining and found that immune function was not impaired in overtrained subjects, as evidenced by no change in IL-6 or T-lymphocyte proliferation. More recently, Halson et al. (73) utilized an overreaching protocol in trained cyclists to simulate overtraining and found that in contrast to the

hypothesis of Smith (225), alterations in plasma cytokines were not related to performance declines associated with overreaching and overtraining. Their 6-week overreaching protocol did produce overreaching/overtraining effects in the cyclists, evidenced by a significant decline in performance as well as disturbances in mood states, and CPK was also elevated. However, there were no changes in TNFα or IL-6, among other hematological measures (73). Clearly, more research is needed to determine the role of cytokines in long-term endurance training.

It is also possible that the mild immunosupression that some investigators have demonstrated in response to training may represent a balance between limiting inflammation from the training stress while maintaining proper immune function (134, 186). For example, NPs appear to be downregulated by strenuous exercise, which may alter host defense capabilities, but this could actually be protective in limiting chronic inflammation (186). Other investigations have reported positive responses to endurance training in terms of NP function and limitation of inflammation. Suzuki et al. (234) reported that increased catecholamine response to strenuous training can suppress NP activity, which may be an adaptive response in order to prevent pathophysiological reactions that could eventually lead to organ damage if inflammation were not attenuated. Thus, limiting the activity of NPs can actually serve to keep the inflammatory response to exercise somewhat in check. However, more longitudinal studies are needed to better elucidate the long-term effects of strenuous endurance training on NP activity, inflammation and other aspects of immune function.

In addition to the direct effects of training on immune cells, there is some evidence that endurance training can deplete glutamine levels. Because this amino acid is a major fuel source for the cells of the immune system, chronic depletion could lead to increased infection in athletes (30). Glutamine is the most abundant free amino acid in both human muscle and plasma. Many investigators have attempted to utilize glutamine as a marker of suppressed immune function and, consequently, indicative of overtraining (73, 180), but the best method of determining the key levels of glutamine in such states has not yet been found (253). In fact, some investigations have found no link between

glutamine and indicators of immune function (80, 121, 252). If it should be determined that glutamine stores decreased by intense endurance exercise have a negative impact on immune function, then supplementing post-daily-exercise training with a CHO plus PRO supplement should allow glutamine levels to be better maintained, presumably leading to better immunity and host protection.

Muscle damage

Muscle damage is a well-documented consequence of strenuous exercise. This damage is due in part to the physical stress placed on the muscle fibers, particularly during the eccentric phase of muscle contraction (35, 56). However, muscle damage also occurs in concentric exercise such as cycling, albeit to a lesser degree, partly due to the rise in cortisol and other catabolic hormones that result in muscle protein degradation (253). Much research has focused on ways to mediate muscle damage and speed the damage recovery process in the hours following exercise. Muscle damage does not just occur during exercise, but can actually continue after exercise for many hours due to a protracted catabolic hormonal milieu, as well as an increase in free radicals and acute inflammation. Not only will such tissue damage limit performance in subsequent exercise bouts due to delayed onset muscle soreness, but it will also compromise the replenishment of muscle glycogen and limit muscle training adaptations (41, 175).

Creatine phosphokinase (CPK, also known as creatine kinase or CK) and myoglobin are blood markers often used as indicators of muscle damage. There are three main isoforms of CPK specific to brain, muscle and heart, and under normal physiological conditions, very little CPK is detected in the blood circulation. Elevated levels indicate damage to muscle (smooth, cardiac or skeletal) or brain, and which type can be assessed by determining which isoform is present. Common clinical reasons for elevated CPK include myocardial infarction, muscle disease, or stroke, but CPK is also an indicator of skeletal muscle damage from strenuous exercise or muscle injury. Athletes have higher levels of serum CPK since strenuous exercise that causes damage to skeletal muscle cells results in increased total serum CPK (23, 147). The highest post-exercise levels are found after prolonged exercise that include eccentric muscular

contractions, such as marathon running, resistance exercise, and downhill running. Total serum CPK activity is markedly elevated for 24 h after the exercise bout and gradually returns to baseline levels with adequate rest and recovery (23).

Another marker of muscle damage is myoglobin, which is found primarily in muscle and in heart. Its heme group is responsible for the red pigmentation of muscle tissues. This protein functions as an intracellular reserve for oxygen, which is stored as oxymyoglobin. This is important as a reserve for prolonged exercise. Myoglobin increases with endurance training, as was first demonstrated in animal studies of dogs, cattle and rats. Lawrie (123) demonstrated increased myoglobin concentration in the muscles of exercised rats, and Pattengale and Holloszy (181) found an 80% increase in myoglobin concentration in leg muscles of endurance run trained rats. *In vitro*, myoglobin also increases the rate of oxygen transport through a fluid layer (222), and likely facilitates oxygen utilization in the skeletal muscle by increasing oxygen transport to the mitochondria (83). In addition to the roles in oxygen storage and transport, myoglobin is important as a marker of muscle damage. When muscle is damaged due to trauma, (ischemia, inflammation, intense exercise), myoglobin is released into the blood and excreted in urine. Clinically, increased myoglobin levels indicate a heart attack or muscle injury. Thus, myoglobin levels are of interest in endurance exercise, primarily due to its function as an indicator of muscle damage in this context.

Significant muscle damage has been demonstrated in various eccentric protocols such as resistance training (11), running (57), reverse cycle pedaling against resistance (241) and stepping protocols (151). Some studies have compared the amounts of measurable muscle damage incurred from eccentric compared to concentric exercise and reported that increases in damage markers were only found in eccentric exercise and not in concentric exercise (25, 186, 226). Sorichter and colleagues (226) found that CPK and myoglobin was significantly increased in protocols of resistance training and downhill running, but not in high-force isokinetic concentric quadriceps contraction exercise. Bruunsgaard et al. (25) used a unique cycling protocol similar to that of Toft et al., (241) described above, in which subjects pedaled normally (concentric contractions) and then

eccentrically against a braked wheel with reversed pedaling revolutions. They found that only the eccentric protocol elicited an increase in CPK (25).

Despite the findings that argue against measurable muscle damage induced by concentric exercise such as cycling, many other investigations have found significant muscle damage using concentric cycling protocols. Konig et al. (117) examined muscle damage markers in professional cyclists after a road race and found that both CPK and myoglobin were significantly increased after the strenuous event. Suzuki et al. (234) also found increases in these two markers when they studied the effects of 3 consecutive days of cycling for 90 min at a moderate intensity in healthy but untrained subjects. Interestingly, the increase was most profound after the first day of cycling, but with the daily progression the levels declined. However, catecholamines increased each day, which appeared to suppress the oxidative function of neutrophils, possibly leading to slight immunosupression (234). Other studies have detected significant increases in CPK after intense cycling (73, 200, 217, 243) as well as in myoglobin after a triathlon (138).

Other investigations have focused on nutritional countermeasures for muscle damage that occurs in cycling exercise and found that compared to CHO alone, CHO+PRO taken during and after exercise decreases muscle damage (39, 209, 210, 217, 219). Saunders et al. (217) found that supplementing with CHO+PRO during and immediately post cycling exercise resulted in only a 2-fold rise in plasma CPK levels, whereas with CHO supplementation alone the CPK levels were increased 14-fold. The team of Saunders et al. (219) recently confirmed this finding using a CHO+PRO gel supplement rather than a beverage. Interestingly, in both of these studies, the CHO+PRO treatment resulted in longer times to fatigue during the cycling performance trial than did the CHO treatment (219). Combest et al. (39) found a similar result of reduced muscle damage but also reported that there is individual variation in the response to CHO+PRO. Nine of the 15 subjects in the Combest et al. (39) study had less CPK with the CHO+PRO treatment while five showed no difference in CPK between the CHO only and the CHO+PRO. The responders had significantly greater times to fatigue than did the CHO+PRO non-responders, and this response did not appear to be influenced by gender

(39). Other studies have shown attenuated CPK levels in response to CHO+PRO compared to isocaloric CHO only treatments without demonstrating a difference in time to exhaustion (209, 210, 246), or no significant difference in both CPK levels and subsequent cycling exercise performance (24, 31). Variations in performance findings will be discussed further below, but protocol differences amongst the studies are likely responsible for differences in time to fatigue data.

CHO+PRO supplementation has been shown to attenuate muscle damage in resistance exercise as well as in endurance exercise. Baty et al. (11) found that supplementing with a CHO+PRO supplement before, during and after exercise limits the muscle damage that occurs during and after resistance exercise training, evidenced by lower levels of CPK and myoglobin than in the placebo alone treatment. Recently, Cockburn and colleagues (36) compared water only, a carbohydrate sports drink, milk and a milk-based CHO+PRO drink in assessing eccentric exercise induced increases in CPK and myoglobin 48 h post-exercise and found that both the milk-based CHO+PRO and the milk only significantly attenuated CPK and myoglobin levels compared to CHO and water. They also reported improved peak torque and total work in an exercise set with the milk-based CHO+PRO and milk compared to CHO and water (Cockburn et al., 2008). In contrast, Wojcik et al. (260) found no change in inflammatory markers or in muscle function after supplementing with a milk-based CHO+PRO recovery versus CHO only beverage following resistance exercise, but did report a trend ($p<0.08$) for lower CPK in the milk-based CHO+PRO treatment (260). While not all investigations show dramatic differences in CHO+PRO and CHO alone, this is likely due to protocol differences.

Long-term training adaptations may occur to help mediate muscle damage. As previously discussed, Suzuki et al. (234) found that levels of CPK declined over three days of training in untrained subjects, but catecholamines increased, potentially leading to slight immunosupression. Three days of training in untrained subjects still represents a relatively acute effect compared to the long-term training undertaken by highly trained endurance athletes. Evans and colleagues (57) compared trained long-distance runners

and untrained healthy subjects and reported chronic elevations in CPK and other inflammatory markers in the trained, yet no acute increases in response to a single intense bout. In contrast, the untrained had a lower baseline level of CPK and other markers, yet had a greater increase after an acute bout (57).

Taken together, most findings in the literature suggest that, with proper supplementation and recovery, adaptations do occur in healthy endurance trained humans such that muscle damage does not become chronic or debilitating (barring injury) and immune function is not severely compromised. However, prospective studies using long-term training have not been undertaken to better understand what the effects of chronic, intense endurance training are across the life span.

INITIATION OF PROTEIN SYNTHESIS

Shifting the post-exercise balance from a muscle-damaged, catabolic hormonal environment, which promotes protein degradation, to one that supports recovery and protein accretion is essential for maximizing training adaptations. Although the muscle can have residual catabolic activity following exercise, it is primed to shift into an anabolic state in the presence of the right nutrients (92). This is due, in part, to an increased sensitivity to insulin post-exercise (95, 97). Insulin is one of the most anabolic hormones in the body, stimulating increases in muscle amino acid (AA) uptake and protein synthesis and reducing protein degradation (17). Rapidly raising the plasma insulin level is key to limiting post-exercise muscle damage and stimulating protein accretion (143, 213). The reason for insulin's important role is that the binding of insulin to its target receptor on the muscle cell activates a complex signaling cascade that ultimately leads to the initiation of protein translation and thus, protein synthesis, as well as glycogen synthesis as discussed previously. Protein synthesis is of great interest, as it is the key adaptation to exercise training and includes the synthesis of oxidative enzymes, contractile fibers, cellular organelles such as mitochondria, membrane receptors, hemoglobin, substrate transporters, and many other important cellular and molecular factors that have key functions related to physical performance.

While exercise (i.e., skeletal muscle contraction) itself stimulates protein synthesis, it also stimulates breakdown of proteins. The balance between degradation and synthesis is mediated in part by the availability of AA. It is becoming clearer that the addition of adequate protein, in particular the essential amino acids (EAA), to a post-exercise CHO supplement is critical for optimizing protein synthesis, creating a positive protein balance, repairing muscle damage, and stimulating training adaptations (44, 62, 67, 118, 127). For example, Cribb and Hayes (44) recently demonstrated that a greater increase in muscle mass and strength could be achieved during 10 wks of resistance training if a CHO+PRO/creatine supplement was provided before and immediately after each daily workout compared with providing the supplement in the morning and at night. Interestingly, when comparing milk versus soy protein ingestion after resistance exercise, it was shown that milk protein promotes muscle protein accretion to a greater extent than soy (256).

As with the process of glycogen synthesis discussed previously, this acute, post-exercise activation of protein synthesis lasts for a limited amount of time (128, 195). Compared to the knowledge of glycogen synthesis pathways, the activation states of the enzymes that control protein translation are less understood. Our laboratory has recently completed several studies addressing the effects of CHO and PRO separately and in combination on the insulin and mTOR signaling pathways. We have found that glycogen synthase, p70S6K, and rpS6 are activated post-exercise in human muscle; however, when subjects receive CHO+PRO post-exercise, activation of glycogen synthase and rpS6 is enhanced, and Akt and mTOR are activated (93). In addition, CHO+PRO supplementation also results in the inhibition of GSK3 (93). As mentioned previously, our laboratory recently demonstrated that, compared to a traditional sports drink, a cereal plus non-fat milk treatment ingested post-exercise lead to greater activation of glycogen synthase and Akt during recovery (110). Our lab also demonstrated in the rat that post-exercise CHO+PRO supplementation increases the rate of muscle glycogen synthesis compared to either CHO or PRO supplementation, and that this is related to an increase in GSK inhibition. Moreover, CHO+PRO was associated with a greater activation (as

indicated by phosphorylation status) of mTOR, rpS6 and inhibition of 4E-BP1 in both red and white skeletal muscle. This is the first study to address the interaction of endurance exercise and the combination of CHO+PRO supplementation on the mTOR signaling pathway (145), and will be discussed in more detail below.

Supplementation with a mixture of essential AA plus CHO has been demonstrated to increase protein synthesis (16, 49, 240). In addition, supplementing with mixtures containing whey, whey plus casein, or CHO+PRO+Leucine has also been demonstrated to stimulate protein synthesis following resistance exercise (118, 137, 239). It is also known that activation of protein synthesis by AA is most responsive immediately after exercise. Raising plasma AA levels post-exercise by infusion or oral supplementation has been shown to transition the muscle from a negative to positive protein balance by stimulating protein synthesis (204). While supplementing with either PRO or AA post-exercise may stimulate protein synthesis, there is increasing evidence that the combination of CHO and either protein or AA can actually have an additive effect (127, 143). This additive effect is likely due in part to the synergist effect that a CHO/AA or CHO+PRO supplement has on the plasma insulin response (227, 266), and the maintenance of an elevated plasma AA profile. Cellularly, this may translate into a greater activation of translation initiation. However, not all studies have demonstrated a difference in insulin response that can be associated with increased protein synthesis. Howarth and colleagues (89) recently assessed whole body net protein balance (WBNB) and muscle fractional synthetic rate (FSR) in response to CHO and CHO+PRO treatments following 2 h of cycling exercise. Six trained subjects were provided either 1.2 g/kg CHO, 1.2 g/kg CHO + 0.4 g PRO, or 1.6 g/kg CHO in random order, and stable isotope tracers were used to measure WBNB, FSR, and protein breakdown over a 4-h recovery period. The investigators found that FSR was significantly greater in the CHO+PRO treatment than either of the CHO treatments, and that WBNB was only increased in the CHO+PRO treatment, due to reduced protein breakdown (89). No differences in plasma glucose or insulin response between treatments were found. Thus, it

is possible that increases in protein synthesis is due not only to elevated insulin, but to the availability of protein as substrate for protein synthesis.

In addition to the many studies utilizing CHO plus different protein types or combinations (whey, casein, EAA, individual AAs, etc.) post-exercise, a recent investigation examined the effects of milk on amino acid uptake and leg protein balance after resistance exercise (53). In this study, fat-free milk (FFM), whole milk (WM) or a greater amount of FFM to match the caloric content of the whole milk was given 1 h after leg resistance exercise. (It should be noted that there was no placebo or CHO-containing treatment, nor another type of protein-containing supplement to compare the three milk treatments against.) While all three treatments increased protein synthesis over baseline, the whole milk treatment demonstrated the greatest increase in amino acid uptake (53). This investigation was the first to examine the effects of a common, natural food (milk) on the protein synthesis response after resistance exercise.

Translation Initiation

Translation initiation, which is the rate-limiting step in translation, is regulated in part by the mammalian target of rapamycin (mTOR) signal-transduction pathway. mTOR is a serine/threonine kinase that integrates signals from nutrients, skeletal muscle contraction and growth factors, functioning as a crucial regulator of protein synthesis. mTOR exists as two signaling complexes: mTOR complex-1 (TORC1) and mTOR complex-2 (TORC2) (139, 215, 216). The TORC1 (mTOR complex 1) consists of mTOR, mLST8 and raptor and is key in activating mRNA translation in response to hormones and growth factors (139, 254). TORC1 promotes translation by phosphorylating two key downstream mTOR effectors, S6K (also known as p70S6 kinase) and the translational repressor 4E-BP1 (eukaryotic initiation factor 4E binding protein 1) (139, 254). Phosphorylation of 4E-BP1 decreases its binding affinity for eIF4E, such that eIF4E is released from 4E-BP1 and can now bind to eIF4G to form the eIF4F pre-initiation complex. This is an essential step in ribosomal assembly (75). This complex now binds to the 40S pre-initiation complex to initiate mRNA translation.

Concurrently, when p70S6K is phosphorylated by mTOR, it then phosphorylates other proteins that are involved in mRNA translation: eIF4B and eIF2kinase, both of which leads to mRNA translation, and most notably, rpS6. Ribosomal protein S6 is associated with enhanced translation of a essential class of mRNAs that have a 5'-terminal oligopyrimidine tract (TOP) at their transcriptional start site, and thus encode for ribosomal proteins as well as elongation factors (7, 54). Because mTOR/TORC1 signaling is so essential to cell growth and protein synthesis, it is important to optimize activation of this complex and downstream targets in order to maximize training adaptations.

It is well established that nutrient availability (and thus, insulin and AA availability), and exercise each activate multiple signal transduction pathways in skeletal muscle, leading to an enhanced rate of protein synthesis. They activate mTOR/TORC1 directly, as well as via the upstream PI3K pathway (21, 113, 139, 215, 216), thereby increasing mRNA translation initiation and protein synthesis after exercise. mTOR is regulated through the sequential activation of a series of upstream kinases including the insulin signaling protein Akt (114). It is well known that the binding of insulin to its skeletal muscle receptors increases phosphorylation of Akt (also known as PKB), which in turn phosphorylates downstream enzymes that ultimately control translation (111, 149, 182, 244). Akt phosphorylates mTOR, which then phosphorylates p70S6K, which activates rpS6. In addition to being an activator of mTOR, Akt in turn inhibits glycogen synthase kinase-3 (GSK3), which is an inhibitor of both glycogen and protein synthesis (45). The inhibition of GSK3 and activation of the enzyme glycogen synthase can also occur via p70S6K activation (5). Therefore, the activation of mTOR via an increase in plasma insulin and AA levels may facilitate an increase in glycogen synthase activity as well as mRNA translation (i.e., increased glycogen synthesis and protein synthesis). This should promote a faster tissue response to training adaptation.

As mentioned previously, a recent investigation by Morrison et al. (145) shed light on the importance of insulin and the signaling pathways that lead to increased mRNA translation. Rats were exercised using a 3-h swimming protocol, and then

provided one of four treatments in solution immediately post-exercise: CHO (23.7% PRO (7.9% w/v), CHO+PRO (23.7% w/v CHO + 7.9% w/v PRO) or placebo (EX). The rats were sacrificed at 0, 30, and 90 min post-exercise, and phosphorylation states of mTOR, p70S6K, rpS6, and 4E-BP1 were analyzed in the red (RQ) and white (WQ) quadriceps. The phosphorylation states of the signaling proteins were transiently increased in all of the treatment groups compared to the placebo/exercise only group. While all the supplements were associated with increased phosphorylation of mTOR and p70S6K after exercise, only CHO+PRO increased rpS6 phosphorylation to a greater extent than the other supplements at 30 min post-exercise, as well as 4E-BP1 at 30 and 90 min post-exercise. The phosphorylation states of rpS6 and 4E-BP1 were highly correlated to insulin concentrations in each group, suggesting a strong relationship between insulin levels and the activation of enzymes critical for mRNA translation. These findings indicate that, compared to CHO or PRO alone, CHO+PRO supplementation may be most effective in activating the mTOR signaling pathway post-exercise (145).

In addition to activation through mTOR as described above, rpS6 can be activated by exercise alone, albeit to a lesser degree. Ivy et al. (93) found increased rpS6 phosphorylation 45 minutes after cycling exercise with placebo and CHO+PRO treatments, although the rpS6 phosphorylation was significantly higher after CHO+PRO compared to the placebo beverage. This suggests that both insulin and exercise effect rpS6 phosphorylation. As discussed previously, Morrison et al. (145) reported a correlation between insulin levels and rpS6 phosphorylation in rats. At 30 min post-exercise, rpS6 phosphorylation was higher in the CHO+PRO treatments compared to the exercise-only group, but interestingly, rpS6 phosphorylation was significantly increased at 90 min post-exercise in the exercised rats that did not receive supplementation. At both the 30 and 90 min time points, insulin was elevated in the three treatment groups compared to the exercised controls. Previous studies have shown that in addition to activation through mTOR, S6K and rpS6 can in fact be activated by exercise through the extracellular signal-regulated kinase 1/2 (ERK1/2) phosphorylation of p90RSK, as well as through the p38 mitogen-activated protein kinase (MAPK) pathway (119, 191, 211,

258). Taken together, these data suggest that exercise alone can increase phosphorylation of rpS6, but that the combined effects of exercise and CHO+PRO supplementation post-exercise can lead to greater phosphorylation of this important protein.

While mTOR is activated by AAs, particularly leucine, either directly or indirectly (20, 116, 201), the process by which this occurs remains to be fully elucidated (115). While mTOR is activated by resistance exercise, (21, 49), it is not directly or indirectly stimulated by aerobic exercise alone (4, 93, 145). Recently, Wang and Proud (255) reported a link between AA and eIF2B activation independent of the mTOR/TORC1 complex. eIF2B is important because it catalyzes the GDP/GTP exchange on eIF2, which is essential for control of ribosomal formation and mRNA translation. They demonstrated that AA deprivation leads to inhibition of eIF2B independent of changes in the phosphorylation status of eIF2, such as would occur in the normal insulin-PI3K signaling pathway. Having established that eIF2B inhibition is independent of TORC1, they identified a new site of phosphorylation on eIF2B. Thus, Wang and Proud reported a novel way to regulate translation initiation through AA (255).

While in many studies, increased plasma insulin correlates with increased protein synthesis, insulin levels are not always increased despite a demonstrated increase in protein synthesis. For example, Gautsch et al. (67) examined in rats the association of eIF4E with 4E-BP1 following recovery from running exercise. The rats were fed wither CHO or a complete meal containing CHO, PRO and fat. Despite having similar insulin responses to both treatments, only in the CHO+PRO+fat treatment stimulated a significant increase in protein synthesis. In addition, increased eIF4E availability was reported to be a key factor in this response (67). The reason for the discrepancies in the insulin response is not known at this time. In the case of the Gautsch et al. (67) study, perhaps providing a meal versus a liquid supplement could account for the difference. A more mechanistic explanation is that there is a critical plasma concentration of insulin required for protein synthesis to occur (60, 63, 68), but it is perhaps only a permissive, limited amount (17, 22, 141). Above this possible threshold insulin level, there may be no additional effect of increased insulin levels. Also, insulin has been shown to have

potentially less of an impact on protein synthesis than on protein degradation (17). Biolo et al. (17) reported that exercise itself (i.e.; muscle contraction) stimulates protein synthesis, and insulin infusion post-exercise reduced protein degradation, and the combined effects resulted in a positive net protein balance. Thus, there appears to be a synergistic effect of the combination of exercise and insulin on increasing protein accretion.

Protein Degradation

Given that the delicate balance of protein synthesis and degradation determines much of the response to acute and chronic exercise, the reduction of post-exercise protein degradation is important for optimized recovery and adaptation to exercise training. Protein degradation, or proteolysis, involves the ubiquitination of proteins targeted for degradation, and their subsequent degradation by the proteasome. This proteolysis pathway is called the Ubiquitin-Proteasome Pathway (UPP), and is considered to be the primary degradation system for most tissues, including skeletal muscle (124, 235). Much of the current literature has reported changes in proteolytic gene expression, with fewer reports of changes in actual content of proteolytic proteins. It is known that key apoptotic genes in the UPP include ubiquitin E3 ligases MuRF-1 and atrogin-1, which share a common critical transcription factor, forkhead box 3A (FOXO3A). FOXO proteins are able to translocate into the nucleus and bind to DNA. Conversely, they can also translocate out of the nucleus upon phosphorylation due to growth factors such as insulin via the Akt pathway (90, 230). When Akt is phosphorylated by components of the insulin/IGF pathway, it translocates into the nucleus and directly phosphorylates FOXO3A on the N-terminus at Ser253, which causes the translocation of FOXO3A out of the nucleus and into the cytoplasm (90, 148). Thus, FOXO3A is unable to carry out its key role in promoting apoptosis because signals associated with cell survival and proliferation have prevailed due to growth factor activation. Few studies to date have examined the role of FOXO3A in exercise models, including resistance exercise (125, 205, 259), and resistance vs running exercise (132). Generally, these studies have verified

a role for FOXO3A in the process of proteolysis following exercise. Therefore, FOXO3A is of current interest in understanding factors that can reduce protein degradation.

Another key aspect of the Ubiquitin-Proteasome Pathway and its regulation post-exercise is ubiquitin itself. This highly conserved, 8.5 kD protein serves to clear away abnormal or damaged proteins by covalently binding to such proteins, targeting them to be degraded by the 26S proteasome (33, 34, 81). Recently, a very few investigations have used total ubiquitin content of Western blot samples as an indicator of protein degradation (51, 197), demonstrating an increase in total ubiquitination in conditions of high proteolysis [injection of oxidative stress-inducing cardiotoxin (197) or hindlimb suspension with or without resistance exercise (51).] These studies suggest that measuring total ubiquitination in tissue samples is a viable way to assess the level at which proteins are targeted for degradation. (It should be noted that, as with any marker of a multi-step biological process, the targeting of a protein for degradation does not necessarily mean that the protein was actually degraded. The final degradation step could be eliminated if the proteasome is malfunctioning. Thus care must be taken when interpreting these types of results.)

In summary, the initiation of protein synthesis is at the heart of endurance training adaptation, and the pathways by which this process is regulated are controlled by post-exercise nutrient availability. Reduction of protein degradation is also essential to hasten recovery and promote adaptation to an exercise stimulus. Whether CHO+PRO supplementation post-exercise will increase the rate of training adaptation to an aerobic exercise stimulus has not been investigated. It is hypothesized that, when provided immediately after each exercise training bout, CHO+PRO will help sustain exercise performance during a progressive exercise training program, activate the mTOR signaling pathway, promote translation initiation and protein synthesis, reduce protein degradation, and bring about a faster rate of aerobic training adaptation than can be achieved when supplementing with CHO alone.

SUBSEQUENT ENDURANCE EXERCISE PERFORMANCE

Studies of subsequent exercise performance (cycling and running) performed after recovering from a previous, strenuous bout have reported significantly increased endurance time to fatigue in the subsequent exercise bout with CHO+PRO supplementation versus CHO alone (173, 217, 238, 257). This improvement in performance was related to a greater recovery of muscle glycogen during the recovery period between the two bouts (257). In addition, our laboratory has reported that supplementing with a CHO+PRO supplement before, during and immediately after resistance exercise reduces muscle tissue damage during as well as in the hours after exercise (11).

Other recent investigations have demonstrated similar performance increases with CHO+PRO compared to CHO alone in subsequent bout exercise (39, 217, 238). As mentioned above regarding during-exercise supplementation, Saunders' team reported an improvement in TTE in an initial, fatiguing cycling bout, as well as in a subsequent intense cycling bout 12-15 h later (217). The subjects consumed the treatments during and immediately after the exercise bouts. In the initial ride to fatigue (75% VO_2peak) subjects rode 29% longer (106.3 +/- 45.2 min) when consuming the CHO+PRO beverage than the CHO beverage (82.3 +/- 32.6 min). In the second ride (85% VO_2peak), subjects performed 40% longer (43.6 +/- 12.5 min) with the CHO+PRO beverage than the CHO beverage (31.2 +/- 8.7 min). These performance improvements occurred despite no differences in RPE, heart rate, blood glucose or blood lactate, but reported significantly reduced muscle damage (as indicated by CPK) in the CHO+PRO treatment (217).

Not all investigations of subsequent endurance exercise performance have reported a benefit with CHO+PRO over CHO alone. A few studies have failed to demonstrate any significant difference in time to fatigue (176, 209, 210, 212) or time trial time (24, 31) in subsequent bouts performed after an initial, fatiguing cycling exercise bout. While the majority of the studies discussed here are performed with trained cyclists or triathletes, a few studies have examined the effects of CHO+PRO on subsequent running performance. Luden et al. (133) investigated the effects of a six-day regimen of CHO+PRO versus CHO post-exercise beverage consumption on muscle damage, muscle

soreness, and race performance in collegiate runners. Of interest to the investigators was how multi-day supplementation might affect performance and damage indicators, since most studies only administer the treatment during or post-exercise. The twenty-three cross-country runners completed their traditional training for six days prior to a cross-country race, and were given a CHO+PRO or CHO only beverage immediately after each training session. Subjects repeated the same protocol with the alternate treatment (6 days of training and supplementation and then racing) following a 21-day washout period. Race performance was assessed using the athlete's finishing ranking, and plasma CPK and muscle soreness were measured. They found that while CPK and muscle soreness were improved with CHO+PRO, there was no significant improvement in cross-country race performance. However, neither group actually demonstrated high levels of muscle damage, so the investigators suggested that the recovery benefits of the CHO+PRO intervention may not have been large enough to cause clear improvements in running performance (133). Furthermore, a field study in which performance is assessed by race placement is inherently flawed, as many additional variables factor into race-day performance than simply glycogen levels or muscle damage levels; race performance is affected by logistical factors and psychological factors as well.

A well-controlled study performed by Niles et al. (173) demonstrated a significant improvement in running TTE in trained endurance runners in response to CHO+PRO compared to an isocaloric CHO treatment. In this study, the beverages were ingested immediately and 1 h after a glycogen depleting exercise bout. The runners then were asked to run to exhaustion at a high intensity (10% above their individual anaerobic threshold). The run time to exhaustion was 21% longer during the CHO+PRO trial (540.7±91.56 sec) than the CHO only trial (446.1±97.09 sec, $p<0.05$). Also, the CHO+PRO treatment resulted in significantly higher serum insulin levels (60.84 versus 30.1 mU/ml) 90 min into recovery than CHO only (173). It appears that the ingestion of a CHO+PRO drink following glycogen-depleting running exercise may speed the recovery process, and improve endurance during a subsequent exercise bout performed on the same day, compared to CHO alone.

Two recent investigations used chocolate milk as a CHO+PRO supplement when assessing the effects of supplementation on a subsequent bout of cycling exercise, which was preceeded hours earlier by an exhaustive cycling bout. Pritchett and colleagues (200) compared CM to a commercially available CHO+PRO supplement. Subjects completed a high-intensity interval exercise protocol, ingested the randomly-ordered supplement immediately post-exercise, and then returned 15-18 h later to perform a performance trial at 85% VO_2max to exhaustion. Time to exhaustion, as well as CPK levels, did not significantly differ between the treatments, suggesting that chocolate milk is just as effective for use as a recovery supplement as a commercially available, more expensive CHO+PRO products (200). An investigation by Thomas and colleagues (238), however, assessed subsequent performance after a 4-h recovery in the lab, during which time subjects were given either CM, a CHO+PRO beverage, or CHO only immediately and 2 h post-exercise. Subjects then cycled to exhaustion at 70% VO_2max. Time to exhaustion was 51% and 43% longer after ingesting CM (32 +/- 11 min) than CHO+PRO (21 +/- 8 min) or CHO (23 +/- 8 min) (238). These results demonstrate that chocolate milk as a CHO+PRO supplement is an effective recovery supplement that can improve subsequent exercise performance.

Possible mechanisms for improved performance following recovery with CHO+PRO supplementation include increased rate of muscle glycogen replenishment, reduced muscle tissue damage, improved immune response and faster muscle tissue repair. With regard to muscle glycogen, its importance as a fuel source during prolonged strenuous exercise cannot be overstated. It has been clearly demonstrated that aerobic endurance is directly related to the initial muscle glycogen stores (14, 15), and that strenuous exercise cannot be maintained once these stores are depleted (1, 14, 15, 78). When provided immediately post-exercise and at 2-h intervals, CHO+PRO supplementation has been found to increase the rate of muscle glycogen synthesis beyond that of both isocarbohydrate and isocaloric CHO supplements, and therefore could contribute substantially to the improved exercise performance following CHO+PRO supplementation (13, 94, 250, 266). It should be noted that the rate of muscle glycogen

synthesis found with a CHO+PRO supplement can be reached with a CHO supplement, but it requires doubling the CHO amount from approximately 0.75g CHO/h to 1.50g CHO/h and increasing the frequency of servings. This is simply not a practical strategy for most athletes.

Combest et al. (39) showed an association between performance and muscle damage by demonstrating not only that TTE is improved with CHO+PRO over CHO alone, but that the cyclists that demonstrated the greatest reduction in muscle damage as assessed by CPK levels also had the greatest TTE improvements. This suggests that potential mechanisms for improved performance may at least in part be due to reductions in muscle damage. In the case of the study by Luden et al. (133) described above, a lack of significant muscle damage may have translated to a lack of opportunity for demonstratable performance improvement with a CHO+PRO beverage.

Thus, the addition of PRO to a CHO supplement appears to increase the efficiency of muscle glycogen synthesis while also conferring the additional advantages of possibly reducing muscle damage, bolstering immune function, and optimizing training adaptation, all while being consumed in appropriate and practical amounts post-exercise.

TRAINING ADAPTATIONS: MAXIMAL OXIDATIVE CAPACITY AND MAXIMAL OXYGEN UPTAKE

Endurance exercise training clearly induces major adaptations in skeletal muscle. Important adaptations that are hallmarks of such training are the increased ability to transport oxygen to the muscle and the increase in the ability of the muscle to utilize the delivered oxygen. The former is a result of increased stroke volume and increased plasma volume, which improve cardiac output. The latter is controlled by the increases in oxidative enzymes and mitochondrial content.

Maximal oxidative capacity

The biochemical adaptations that occur in response to endurance training were first reported in 1967 by John Holloszy in his classic study of treadmill running in rats

(84). The rats ran twice daily, 5 days a week for 12 weeks, and the workload progressively increased over the 12-week period. Initially their bouts were 10 min at 22 meters/min, and by the end of the 12 weeks, had increased to 120 min of continuous running at 31 meters/min, with 12 30-sec higher intensity sprints included in the run. Tissue analysis revealed a two-fold increase in the activity of many mitochondrial enzymes. In addition, the fraction of homogenate that was mitochondrial protein was increased by 60% (84). Interestingly, while some mitochondrial enzymes increase two-fold, others may only increase 35% to 60%, and still others may not increase at all (83). (This accounts for the reason that mitochondrial protein increased 60% and not closer to two-fold.) A few years later, Benzi et al. (12) showed in a rat model that mitochondrial enzymes were modulated by workload and training time such that increases in those variables correlated with increased enzyme activity. In addition, if training level decreased, enzymatic activity also decreased proportionally (12).

Two key enzymes that are indicative of increased muscle oxidative capacity are citrate synthase and succinate dehydrogenase (SDH), both of which are found in the mitochondria. Citrate synthase (CS) is found in most all living cells, playing a key role in the citric acid cycle (or Krebs cycle). CS is synthesized via cytoplasmic ribosomes, then transported into the mitochondrial matrix. As such, it is frequently used as an enzymatic marker of mitochondrial density and aerobic capacity of the muscle. As a key player in the first step of the citric acid cycle, it is inhibited by high ATP:ADP ratios, as well as high ratios of acetyl-CoA:CoA, and NADH:NAD, because high concentrations of ATP, acetyl-CoA, and NADH mean that the cell's energy supply is sufficient for the energy demands of the moment. CS is also inhibited by its product, citrate, as well as succinyl CoA, a downstream citric acid cycle intermediate. Succinate dehydrogenase (SDH) is also a key mitochondrial enzyme that catalyzes the oxidation of succinate to fumarate in the Krebs cycle, and as such is often used as a marker of Krebs cycle activity. Because it is bound to the mitochondrial membrane, it is also used as an index of mitochondrial protein amount. Thus, like CS, SDH is also a well-established marker of oxidative capacity of the muscle. Both CS and SDH have been demonstrated to increase in

response to endurance training.

The increase in CS in response to running in rats has been established for quite some time (9, 87). Many studies use it as a marker of increased muscle oxidative capacity, and it is increased in response to endurance training in humans (27, 61). SDH has also been shown to increase after an endurance training program in both rats (84, 207) and humans (47, 77, 146, 203). The benefit of increases in such enzyme activity is that it leads to less homeostatic disturbance in exercise of the same given intensity in the trained versus untrained state. When considering this homeostatic balance, it is easy to understand why important training adaptations to regularly performed endurance exercise training include a slower rate of muscle glycogen utilization, increased reliance on fat oxidation and decreased lactate production during exercise of a given intensity (86).

In addition to the two key enzymes discussed above, another protein of interest has direct applicability to training adaptations: the transcription coactivator peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α). This protein is of interest because of its role in mitochondrial biogenesis. One of the key adaptations to endurance exercise training is the increase in skeletal muscle mitochondria (84, 85). Mitochondrial biogenesis involves complex coordination of expression of the mitochondrial genome, as well as nuclear genes that encode mitochondrial proteins, and PGC-1α plays a key role in this elaborate process (112, 129, 202). PGC-1α is a transcriptional coactivator of transcription factor PPARγ and together, they regulate the expression of genes that encode mitochondrial proteins. It is known that a single bout of exercise or stimulated skeletal muscle contraction induces an increase in both PGC-1α mRNA and protein in skeletal muscle (8, 71, 91, 237). In addition to the concomitant increases in PGC-1α and mitochondria in response to exercise, transgenic overexpression studies in animal models have also shown that increasing PGC-1α content induces an increase in mitochondria (130, 262).

Another mechanism though which PGC-1α mediates training adaptations may be through its role in fiber type determination. PGC-1α is preferentially expressed in Type I (slow) skeletal muscle fibers, but Lin and colleagues (130) demonstrated that when PGC-

1α is expressed at physiological levels in transgenic mice, fiber type conversion occurs such that the muscles normally rich in the Type II phenotype becomes much more like Type I fibers (130). The fibers show greater fatigue resistance, express more genes of oxidative metabolism, and contain more myoglobin, all of which are normally characteristics of Type I fibers (130).

The importance of PGC-1α regulating oxidative metabolism was demonstrated by Leone and colleague (126), who developed a knockout mouse model that could be studied across the life span from birth to older age. Although the knockout mice (PGC-1α -/-) were viable, they had many significant systemic abnormalities, all stemming from an abnormal metabolic phenotype. Compared to normal mice, as the knockout mice aged, they expressed more lipogenic genes, displayed an abnormal increase in body fat, developed fewer mitochondria and had a lower respiratory capacity which resulted in reduced exercise capacity. Other abnormalities found in the knockouts included an impaired ability to thermoregulate, the development of hepatic steatosis (fatty liver), and development of vacuolar lesions in the central nervous system (126). These findings underscore the key role of PGC-1α in the ability to adapt to a host of metabolic and physiological stressors such as are encountered during endurance exercise training.

In summary, PGC-1α clearly has an important role in regulating adaptations to endurance exercise training. While the adaptive increase in mitochondria may initially be due to increased PGC-1α activation prior to the actual increase in the amount of PGC-1α protein content (261), the increase in PGC-1α protein itself appears to sustain and enhance the increased mitochondrial biogenesis (261). Therefore, an increase in PGC-1α protein content in skeletal muscle in response to endurance exercise training can be an indicator of the level of adaptive response to the exercise training. To our knowledge, the increase in PGC-1α protein in response to any type of nutritional supplementation post-exercise during an endurance training program has not been investigated. However, it is reasonable to hypothesize that the greatest amount of increase in PGC-1α protein in the skeletal muscle of exercisers will occur in those who are able to recover better after daily training sessions and possibly adapt faster to their training stimulus.

Maximal oxygen consumption

A signature of endurance training is a high VO_2max – the ability to use a great amount of oxygen during endurance exercise. It is common for professional cyclists or triathletes to have VO_2max values in the ranges of 75-85+ ml/kg/min for men and 65 – 75 ml/kg/min for women. Highly trained or elite non–professionals will usually fall between 55-75 ml/kg/min for men and 45-65 ml/kg/min for women. For comparison purposes, sedentary but healthy individuals are commonly in the range of 30-40 ml/kg/min, 30-35 ml/kg/min for women. The more oxygen an individual is able to utilize, the longer and harder the athlete can exercise, all else being equal (hydration status, nutritional status, glucose availability, etc.).

The increase in VO_2max that occurs in response to undertaking an endurance training program is caused by two key physiological elements: stroke volume (SV) and oxygen extraction (arteriovenous difference, or a-vO_2). The three elements that lead to increased SV are (1) increases in plasma volume, venous return and left ventricular volume, which together contribute to increased end diastolic volume (EDV), (2) increased myocardial contractility, and (3) decreased total peripheral resistance (TPR) (198). The increased ability of the muscle to extract oxygen, quantified as the arteriovenous difference (a-vO_2), is due to two elements, listed in order of importance: (1) increased capillary density, and (2) increased mitochondrial density in the muscle (198). In young, sedentary but healthy individuals, these two elements are relatively equal determinants of increasing VO_2max (198).

Classic studies by Saltin et al. (214) and Ekblom et al. (52) demonstrated that increases in VO_2max are due to increases in maximal cardiac output (CO) as well as an increased a-vO_2 difference. Saltin et al. (214) studied 5 males after 20 days of bed rest and 55 days of training. Three of the subjects were previously sedentary, while the other two were previously active but not trained individuals. In the three previously sedentary subjects, CO increased by 16.5% after training, and the a-vO_2 difference also increased by 16.5%. Thus, the improvements were equally attributed to increased CO – entirely due

to increased stroke volume (SV) – and increased a-vO_2 difference (214). However, in the previously trained subjects, the changes in the above variables were less dramatic, indicating that with lower initial maximal oxygen uptake levels the changes were more pronounced. Ekblom et al. (52) trained eight male subjects for 16 weeks, and found a 16.2% increase in VO_2max, mainly due to increased SV. The a-vO_2 difference after training was also significantly improved, and thus they concluded that the increase in VO_2max was partly due to increased SV and CO, and partly due to the increased a-vO_2 difference (52).

The time course for increases in maximal oxygen consumption is somewhat controversial, and depends upon many factors including exercise mode, frequency, duration, intensity, and the initial fitness level of the individual. Two separate studies published in 1977 examined the time course for VO_2max increases in response to endurance training. Henriksson and Reitman (77) trained 13 healthy men on cycle ergometers for 20 min per day for four days a week over an 8-week period. The investigators increased the work load over the course of the training period in order to keep the subjects working at a relative rate of 80% VO_2max. They examined VO_2max, as well as SDH activity in the vastus lateralis. All three variables had significantly increased after only three weeks of training. After 8 weeks, VO_2max had increased 19% over pre-training levels, and SDH activity increased 32% (77). Then, they had the subjects detrain for six weeks before reassessing the above variables. While VO_2max was still 16% above the pre-training levels and not significantly different from the value achieved at the end of the 8-week training period, SDH activity had returned to pre-training levels, indicating that improvements in VO_2max are independent of, but associated with, skeletal muscle oxidative capacity adaptation (77). It is likely that increased muscle oxidative capacity is more important in submaximal endurance exercise than in higher intensity work loads (77, 84).

In a classic study examining the effects of frequent, intense exercise on improvements in VO_2max, Hickson et al. (79) trained eight subjects for 6 days per week for 10 weeks. Subjects cycled on an ergometer three days per week, performing six 5-

minute intervals during which VO_2max was attained in each interval. As the subject's work capacity increased over the training period, the bike work load was also increased to elicit their maximal VO_2 during each interval. The other three days, they ran as fast as possible on a treadmill for 30 minutes per session in the first week, 35 minutes per session in the second week, and then 40 or more minutes per session thereafter. After only one week of training, the group average VO_2max had increased by 5%. The total average increase after the 10 week period was ~40% (79). Interestingly, the increase in VO_2max over the 10 week period was linear without indication of leveling off (79).

Other investigators have shown a 17% increase in VO_2peak for untrained males and 22% in untrained females after 7 weeks of endurance training (27), while some have shown a 10% improvement in VO_2peak after a 31-day training period (194). Spina et al. (228) found a 9% VO_2peak increase after only 7-10 days of training. It is important to note that all of these improvements are in healthy, active but untrained subjects and thus demonstrate higher and more rapid improvements than would moderately trained individuals. For example, Hickson et al. (79) found that the largest increase in VO_2max, ~53%, was evidenced in the two subjects with the lowest initial oxygen uptake values (79).

In their 31-day training protocol, Phillips et al. (194) had 7 untrained subjects cycle for 2 h/day 5-6 days/week at 59% VO_2peak. They found lower exercise blood lactate concentration, phosphocreatine hydrolysis and glycogen depletion after only the first 5 days of training, and after 31 days of training, these variables were lower still than at baseline. They reported that muscle oxidative potential (estimated from succinate dehydrogenase activity) was not increased until the end of the 31 day period. Therefore, they concluded that some characteristic adaptations such as lower blood lactate and glycogen depletion occur rapidly and precede that of the mitochondrial adaptations that would lead to greater oxygen utilization (194). In contrast, however, Spina et al. (228) reported that the adaptive increase in mitochondrial enzymes occurs much more quickly than previously suggested. In their study, twelve untrained subjects cycled 2 h/day for 7 or 10 days, as referenced above, at 60% - 70% of VO_2peak. VO_2peak increased after this

period by 9% and blood lactate levels were lower at the same absolute work load as before training. CS and other mitochondrial enzymes were increased by approximately 30%, providing evidence that shorter term training periods produce a significant adaptive response in mitochondrial enzymes. A recent study by Green et al. (72) demonstrated an increase in glucose and lactate transporters after only 3 days of cycling training in untrained subjects, but no change was found in HK. Clearly, metabolic adaptations occur quickly in response to endurance training, with the enzymes involved in glucose uptake and lactate metabolism likely increasing more quickly than the oxidative enzymes. However, it would appear that in untrained populations, muscle oxidative adaptations can occur in 7 to 10 days (228).

As rapidly as these adaptations can occur, they can also be lost quickly as well if training ceases altogether. This was demonstrated in the study by Henriksson and Reitman (77), discussed above, as VO_2max improvements were maintained after 6 weeks of detraining, whereas SDH activity regressed back to pre-training levels (Henriksson and Reitman (77). In a classic study of the effects of 84 days of detraining in trained endurance runners and cyclists, Coyle et al. (43) found that VO_2max decreased by 7% over the first 12 days of detraining, and stabilized at 56 days of detraining at 16% below their trained level. The initial decline of 7% in VO_2max was related to a reduced SV and the later decline, an additional 9%, in VO_2max was due to reduced a-vO_2 difference. Cardiac output, estimated from heart rate and SV, decreased by 8% over 21 days but then stabilized to remain at 8-9% below trained levels for the rest of the detraining period. CS and SDH activity decreased 17.1% and 18.5%, respectively, in 12 days, and dropped to 39.6% and 32.5%, respectively, below trained levels at the end of the 84 day period (43). While CS and SDH decreased, they still remained 50% higher than levels measured in a group of eight untrained, sedentary controls, and the a-vO_2 difference and VO_2max, as well as muscle capillarization, were still higher than in the control subjects after 84 days of detraining (43).

Given what is known about increasing maximal and peak oxygen consumption through endurance training, how might CHO+PRO supplementation affect this outcome?

Okazaki and colleagues (174) recently compared the effects of a CHO+PRO supplement to a placebo supplement in a model of chronic exercise training older male subjects who cycled for 60 min/d, 3 d/wk for 8 wk at 60-75% VO2peak and ingested either CHO+PRO or placebo immediately post-exercise each session. They reported a two-fold increase in VO2max in the CHO+PRO group compared to the placebo group with significant stroke volume and plasma volume increases only found in the CHO+PRO group (174). Thus, nutritional supplementation may increase the magnitude of training adaptations compared to the exercise stimulus alone. It should be noted, however, that the investigation by Okazaki and colleagues compared CHO+PRO supplementation to placebo only, and did not include any other energy-containing treatment. In the training studies of training adaptations proposed here, we extend these findings by including an isocaloric CHO treatment in addition to a CHO+PRO supplement and placebo.

Adaptations in terms of increased lean mass and muscle hypertrophy after 12 weeks of resistance training and supplementing with milk versus soy protein have been reported (76), and Flakoll et al. (62) reported that CHO+PRO supplementation during a 54-day basic training period was associated with improved immune function, decreased muscle soreness and increased hydration status, but these investigations are not entirely applicable to what will occur with endurance training. Luden et al. (133) examined a 6-day CHO+PRO or CHO regimen in runners and reported improved muscle soreness and lower CPK levels in the CHO+PRO treatment, but the subjects were already well trained and the exercise stimulus was not sufficient to produce abnormally high levels of muscle damage in this population. However, it is reasonable to hypothesize that supplementing post-exercise with CHO+PRO will lead to a faster rate of training adaptations by promoting protein synthesis, by improving recovery, and allowing for a faster progression in training stimulus.

SUMMARY

Acute endurance exercise performance, post-exercise recovery, and adaptation to endurance exercise training are highly complex processes that involve the integration of multiple systems and many components that are positively affected by proper post-

exercise nutrition. Compared to CHO supplements alone, CHO+PRO supplementation during endurance exercise can increase time to exhaustion. Post-exercise CHO+PRO supplementation increases the rate of recovery from prolonged endurance exercise that depletes muscle glycogen stores and improves subsequent exercise performance. In addition, CHO+PRO supplementation has been shown to increase muscle glycogen synthesis, reduce muscle damage, and activate cellular signaling proteins that control both glycogen synthesis and initiation of mRNA translation for protein synthesis. Furthermore, a CHO+PRO supplement post-exercise has been found to increase protein synthesis to a greater degree than CHO, PRO or AA supplementation alone. While it has not previously been investigated, we hypothesize that CHO+PRO supplementation also can protect immune function during periods of intense training and recovery, increase the rate of improvement in VO_2max and muscle oxidative capacity during an endurance training period, and improve body composition. In summary, CHO+PRO supplementation is a viable strategy to prove endurance exercise performance and increase the rate of endurance training adaptations.

REFERENCES

1. Ahlborg B, Bergstrom J, Ekelund LG, and Hultman E. Muscle glycogen and muscle electrolytes during prolonged physical exercise. Acta Physiol Scand 70: 129-142, 1967.
2. Akerstrom T, Steensberg A, Keller P, Keller C, Penkowa M, and Pedersen BK. Exercise induces interleukin-8 expression in human skeletal muscle. J Physiol 563: 507-516, 2005.
3. Akira S, Taga T, and Kishimoto T. Interleukin-6 in biology and medicine. Adv Immunol 54: 1-78, 1993.
4. Anthony TG, McDaniel BJ, Knoll P, Bunpo P, Paul GL, and McNurlan MA. Feeding Meals Containing Soy or Whey Protein after Exercise Stimulates Protein Synthesis and Translation Initiation in the Skeletal Muscle of Male Rats. J Nutr 137: 357-362, 2007.
5. Armstrong JL, Bonavaud SM, Toole BJ, and Yeaman SJ. Regulation of Glycogen Synthesis by Amino Acids in Cultured Human Muscle Cells. J Biol Chem 276: 952-956, 2001.
6. Arnold L, Henry A, Poron F, Baba-Amer Y, van Rooijen N, Plonquet A, Gherardi RK, and Chazaud B. Inflammatory monocytes recruited after skeletal muscle injury switch into antiinflammatory macrophages to support myogenesis. J Exp Med 204: 1057-1069, 2007.
7. Avni D, Biberman Y, and Meyuhas O. The 5' terminal oligopyrimidine tract confers translational control on TOP mRNAs in a cell type- and sequence context-dependent manner. Nucleic Acids Res 25: 995-1001, 1997.
8. Baar K, Wende AR, Jones TE, Marison M, Nolte LA, Chen MAY, Kelly DP, and Holloszy JO. Adaptations of skeletal muscle to exercise: rapid increase in the transcriptional coactivator PGC-1. FASEB J 16: 1879-1886, 2002.
9. Baldwin KM, Klinkerfuss GH, Terjung RL, Mole PA, and Holloszy JO. Respiratory capacity of white, red, and intermediate muscle: adaptative response to exercise. Am J Physiol -- Legacy Content 222: 373-378, 1972.
10. Bartholomew K, O'Brien BJ, and Gill ND. Plasma volume expansion 24-hours post-exercise: effect of doubling the volume of replacement fluid. Journal of Sports Science and Medicine 4: 179-184, 2005.
11. Baty JJ, Hwang H, Ding Z, Bernard JR, Wang B, Kwon B, and Ivy JL. The effect of a carbohydrate and protein supplement on resistance exercise performance, hormonal response, and muscle damage. J Strength Cond Res 21: 321–329, 2007.
12. Benzi G, Panceri P, de Bernardi M, Villa R, Arcelli E, D'Angelo L, Arrigoni E, and Berte F. Mitochondrial enzymatic adaptation of skeletal muscle to endurance training. J Appl Physiol 38: 565-569, 1975.
13. Berardi JM, Price TB, Noreen EE, and Lemon PW. Postexercise muscle glycogen recovery enhanced with a carbohydrate-protein supplement. Med Sci Sports Exerc 38: 1106-1113, 2006.
14. Bergstrom J, Hermansen L, Hultman E, and Saltin B. Diet, muscle glycogen and physical performance. Acta Physiol Scand 71: 140-150, 1967.

15. Bergström J, and Hultman E. Muscle glycogen synthesis after exercise: an enhancing factor localized to the muscle cells in man. Nature 210: 309-310, 1966.
16. Biolo G, Tipton KD, Klein S, and Wolfe RR. An abundant supply of amino acids enhances the metabolic effect of exercise on muscle protein. Am J Physiol Endocrinol Metab 273: E122-129, 1997.
17. Biolo G, Williams BD, Fleming RY, and Wolfe RR. Insulin action on muscle protein kinetics and amino acid transport during recovery after resistance exercise. Diabetes 48: 949-957, 1999.
18. Blannin AK, Chatwin LJ, Cave R, and Gleeson M. Effects of submaximal cycling and long-term endurance training on neutrophil phagocytic activity in middle aged men. Br J Sports Med 30: 125-129, 1996.
19. Blom P, Høstmark A, Vaage O, Kardel K, and Maehlum S. Effect of different post-exercise sugar diets on the rate of muscle glycogen synthesis. Med Sci Sports Exc 19: 491-496, 1987.
20. Blomstrand E, Eliasson J, Karlsson HKR, and Kohnke R. Branched-Chain Amino Acids Activate Key Enzymes in Protein Synthesis after Physical Exercise. J Nutr 136: 269S-273, 2006.
21. Bolster DR, Jefferson LS, and Kimball SR. Regulation of protein synthesis associated with skeletal muscle hypertrophy by insulin-, amino acid- and exercise-induced signalling. Proc Nutr Soc 63: 351-356, 2004.
22. Borsheim E, Cree MG, Tipton KD, Elliott TA, Aarsland A, and Wolfe RR. Effect of carbohydrate intake on net muscle protein synthesis during recovery from resistance exercise. J Appl Physiol 96: 674-678, 2004.
23. Brancaccio P, Maffulli N, and Limongelli FM. Creatine kinase monitoring in sport medicine. Br Med Bull 81-82: 209-230, 2007.
24. Breen L, Tipton KD, and Jeukendrup AE. No effect of carbohydrate-protein on cycling performance and indices of recovery. Med Sci Sports Exerc 42: 1140-1148, 2010.
25. Bruunsgaard H, Galbo H, Halkjaer-Kristensen J, Johansen TL, MacLean DA, and Pedersen BK. Exercise-induced increase in serum interleukin-6 in humans is related to muscle damage. J Physiol 499: 833-841, 1997.
26. Carrithers JA, Williamson DL, Gallagher PM, Godard MP, Schulze KE, and Trappe SW. Effects of postexercise carbohydrate-protein feedings on muscle glycogen restoration. J Appl Physiol 88: 1976-1982, 2000.
27. Carter SL, Rennie CD, Hamilton SJ, and Tarnopolsky MA. Changes in skeletal muscle in males and females following endurance training. Can J Physiol Pharmacol 79: 386-392, 2001.
28. Casey A, Short AH, Hultman E, and Greenhaff PL. Glycogen resynthesis in human muscle fibre types following exercise-induced glycogen depletion. J Physiol 483: 265-271, 1995.
29. Cassatella MA, Meda L, Gasperini S, Calzetti F, and Bonora S. Interleukin 10 (IL-10) upregulates IL-1 receptor antagonist production from lipopolysaccharide-stimulated human polymorphonuclear leukocytes by delaying mRNA degradation. J Exp Med 179: 1695-1699, 1994.

30. Castell LM, Poortmans JR, and Newsholme EA. Does glutamine have a role in reducing infections in athletes? Eur J Appl Physiol Occup Physiol 73: 488-490, 1996.
31. Cermak NM, Solheim AS, Gardner MS, Tarnopolsky MA, and Gibala MJ. Muscle metabolism during exercise with carbohydrate or protein-carbohydrate ingestion. Med Sci Sports Exerc 41: 2158-2164, 2009.
32. Chernoff AE, Granowitz EV, Shapiro L, Vannier E, Lonnemann G, Angel JB, Kennedy JS, Rabson AR, Wolff SM, and Dinarello CA. A randomized, controlled trial of IL-10 in humans. Inhibition of inflammatory cytokine production and immune responses. J Immunol 154: 5492-5499, 1995.
33. Ciechanover A. The ubiquitin-proteasome proteolytic pathway. Cell 79: 13-21, 1994.
34. Ciechanover A, and Schwartz AL. The ubiquitin-mediated proteolytic pathway: mechanisms of recognition of the proteolytic substrate and involvement in the degradation of native cellular proteins. FASEB J 8: 182-191, 1994.
35. Clarkson PM, and Hubal MJ. Exercise-induced muscle damage in humans. Am J Phys Med Rehabil 81: S52-S69, 2002.
36. Cockburn E, Hayes PR, French DN, Stevenson E, and Gibson ASC. Acute milk-based protein-CHO supplementation attenuates exercise-induced muscle damage. Applied Physiology, Nutrition & Metabolism 33: 775-783, 2008.
37. Coggan AR, and Coyle EF. Effect of carbohydrate feedings during high-intensity exercise. J Appl Physiol 65: 1703-1709, 1988.
38. Coggan AR, and Coyle EF. Reversal of fatigue during prolonged exercise by carbohydrate infusion or ingestion. J Appl Physiol 63: 2388-2395, 1987.
39. Combest T, Saunders M, Kane M, and Todd K. Attenuated CPK Following Carbohydrate/protein Intervention Improves Subsequent Performance. Med Sci Sports Exc 37: S42, 2005.
40. Convertino VA. Blood volume: its adaptation to endurance training. Med Sci Sports Exerc 23: 1338-1348, 1991.
41. Costill DL, Pascoe DD, Fink WJ, Robergs RA, Barr SI, and Pearson D. Impaired muscle glycogen resynthesis after eccentric exercise. J Appl Physiol 69: 46-50, 1990.
42. Coyle EF, Hemmert MK, and Coggan AR. Effects of detraining on cardiovascular responses to exercise: role of blood volume. J Appl Physiol 60: 95-99, 1986.
43. Coyle EF, Martin WH, 3rd, Sinacore DR, Joyner MJ, Hagberg JM, and Holloszy JO. Time course of loss of adaptations after stopping prolonged intense endurance training. J Appl Physiol 57: 1857-1864, 1984.
44. Cribb PJ, and Hayes A. Effects of supplement timing and resistance exercise on skeletal muscle hypertrophy. Med Sci Sports Exerc 38: 1918-1925, 2006.
45. Cross DA, Alessi DR, Cohen P, Andjelkovich M, and Hemmings BA. Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase B. Nature 378: 785-789, 1995.
46. Currell K, and Jeukendrup AE. Superior endurance performance with ingestion of multiple transportable carbohydrates. Med Sci Sports Exerc 40: 275-281, 2008.

47. De Bock K, Derave W, Eijnde BO, Hesselink MKC, Koninckx E, Rose AJ, Schrauwen P, Bonen A, Richter EA, and Hespel PJ. Effect of training in the fasted state on metabolic responses during exercise with carbohydrate intake. J Appl Physiol 01195.02007, 2008.
48. Doyle JA, Sherman WM, and Strauss RL. Effects of eccentric and concentric exercise on muscle glycogen replenishment. J Appl Physiol 74: 1848-1855, 1993.
49. Dreyer HC, Drummond MJ, Pennings B, Fujita S, Glynn EL, Chinkes DL, Dhanani S, Volpi E, and Rasmussen BB. Leucine-enriched essential amino acid and carbohydrate ingestion following resistance exercise enhances mTOR signaling and protein synthesis in human muscle. Am J Physiol Endocrinol Metab 294: E392-400, 2008.
50. Dufaux B, and Order U. Complement activation after prolonged exercise. Clin Chim Acta 179: 45-49, 1989.
51. Dupont-Versteegden EE, Fluckey JD, Knox M, Gaddy D, and Peterson CA. Effect of flywheel-based resistance exercise on processes contributing to muscle atrophy during unloading in adult rats. J Appl Physiol 101: 202-212, 2006.
52. Ekblom B, Astrand PO, Saltin B, Stenberg J, and Wallstrom B. Effect of training on circulatory response to exercise. J Appl Physiol 24: 518-528, 1968.
53. Elliot TA, Cree MG, Sanford AP, Wolfe RR, and Tipton KD. Milk ingestion stimulates net muscle protein synthesis following resistance exercise. Med Sci Sports Exerc 38: 667-674, 2006.
54. Escobar J, Frank JW, Suryawan A, Nguyen HV, Kimball SR, Jefferson LS, and Davis TA. Physiological rise in plasma leucine stimulates muscle protein synthesis in neonatal pigs by enhancing translation initiation factor activation. Am J Physiol Endocrinol Metab 288: E914-921, 2005.
55. Espersen GT, Elbaek A, Ernst E, Toft E, Kaalund S, Jersild C, and Grunnet N. Effect of physical exercise on cytokines and lymphocyte subpopulations in human peripheral blood. APMIS 98: 395-400, 1990.
56. Evans WJ. Effects of Exercise on Senescent Muscle. Clin-Orthop 403 Suppl: S211-220, 2002.
57. Evans WJ, Meredith CN, Cannon JG, Dinarello CA, Frontera WR, Hughes VA, Jones BH, and Knuttgen HG. Metabolic changes following eccentric exercise in trained and untrained men. J Appl Physiol 61: 1864-1868, 1986.
58. Febbraio MA, Hiscock N, Sacchetti M, Fischer CP, and Pedersen BK. Interleukin-6 Is a Novel Factor Mediating Glucose Homeostasis During Skeletal Muscle Contraction. Diabetes 53: 1643-1648, 2004.
59. Febbraio MA, and Pedersen BK. Muscle-derived interleukin-6: mechanisms for activation and possible biological roles. FASEB J 16: 1335-1347, 2002.
60. Fedele MJ, Hernandez JM, Lang CH, Vary TC, Kimball SR, Jefferson LS, and Farrell PA. Severe diabetes prohibits elevations in muscle protein synthesis after acute resistance exercise in rats. J Appl Physiol 88: 102-108, 2000.
61. Fernstrom M, Tonkonogi M, and Sahlin K. Effects of acute and chronic endurance exercise on mitochondrial uncoupling in human skeletal muscle. J Physiol 554: 755-763, 2004.

62. Flakoll PJ, Judy T, Flinn K, Carr C, and Flinn S. Postexercise protein supplementation improves health and muscle soreness during basic military training in marine recruits. J Appl Physiol 96: 951-956, 2004.
63. Fluckey JD, Vary TC, Jefferson LS, and Farrell PA. Augmented insulin action on rates of protein synthesis after resistance exercise in rats. Am J Physiol Endocrinol Metab 270: E313-319, 1996.
64. Frydelund-Larsen L, Penkowa M, Akerstrom T, Zankari A, Nielsen S, and Pedersen BK. Muscle: Exercise induces interleukin-8 receptor (CXCR2) expression in human skeletal muscle. Exp Physiol 92: 233-240, 2007.
65. Gabriel H, and Kindermann W. The acute immune response to exercise: what does it mean? Int J Sports Med 18: S28-45, 1997.
66. Gabriel H, Urhausen A, and Kindermann W. Circulating leucocyte and lymphocyte subpopulations before and after intensive endurance exercise to exhaustion. Eur J Appl Physiol Occup Physiol 63: 449-457, 1991.
67. Gautsch TA, Anthony JC, Kimball SR, Paul GL, Layman DK, and Jefferson LS. Availability of eIF4E regulates skeletal muscle protein synthesis during recovery from exercise. Am J Physiol Cell Physiol 274: C406-414, 1998.
68. Gingras A-C, Gygi SP, Raught B, Polakiewicz RD, Abraham RT, Hoekstra MF, Aebersold R, and Sonenberg N. Regulation of 4E-BP1 phosphorylation: a novel two-step mechanism. Genes & Dev 13: 1422-1437, 1999.
69. Gleeson M. Immune function in sport and exercise. J Appl Physiol 103: 693-699, 2007.
70. Gleeson M. The scientific basis of practical strategies to maintain immunocompetence in elite athletes. Exercise Immunology Review 6: 75-101, 2000.
71. Goto M, Terada S, Kato M, Katoh M, Yokozeki T, Tabata I, and Shimokawa T. cDNA Cloning and mRNA analysis of PGC-1 in epitrochlearis muscle in swimming-exercised rats. Biochem Biophys Res Commun 274: 350-354, 2000.
72. Green HJ, Bombardier EB, Duhamel TA, Stewart RD, Tupling AR, and Ouyang J. Metabolic, Enzymatic and Transporter Responses in Human Muscle During Three Consecutive Days of Exercise and Recovery. Am J Physiol Regul Integr Comp Physiol 00171.02008, 2008.
73. Halson SL, Lancaster GI, Jeukendrup AE, and Gleeson M. Immunological responses to overreaching in cyclists. Med Sci Sports Exerc 35: 854-861, 2003.
74. Hansen JB, Wilsgard L, and Osterud B. Biphasic changes in leukocytes induced by strenuous exercise. Eur J Appl Physiol Occup Physiol 62: 157-161, 1991.
75. Hara K, Yonezawa K, Kozlowski MT, Sugimoto T, Andrabi K, Weng Q-P, Kasuga M, Nishimoto I, and Avruch J. Regulation of eIF-4E BP1 Phosphorylation by mTOR. J Biol Chem 272: 26457-26463, 1997.
76. Hartman JW, Tang JE, Wilkinson SB, Tarnopolsky MA, Lawrence RL, Fullerton AV, and Phillips SM. Consumption of fat-free fluid milk after resistance exercise promotes greater lean mass accretion than does consumption of soy or carbohydrate in young, novice, male weightlifters. Am J Clinical Nutrition 86: 373-381, 2007.

77. Henriksson J, and Reitman JS. Time course of changes in human skeletal muscle succinate dehydrogenase and cytochrome oxidase activities and maximal oxygen uptake with physical activity and inactivity. Acta Physiol Scand 99: 91-97, 1977.
78. Hermansen L, Hultman E, and Saltin B. Muscle glycogen during prolonged severe exercise. Acta Physiol Scand 71: 129-139, 1967.
79. Hickson RC, Bomze HA, and Holloszy JO. Linear increase in aerobic power induced by a strenuous program of endurance exercise. J Appl Physiol 42: 372-376, 1977.
80. Hiscock N, and Pedersen BK. Exercise-induced immunodepression- plasma glutamine is not the link. J Appl Physiol 93: 813-822, 2002.
81. Hochstrasser M. Ubiquitin, proteasomes, and the regulation of intracellular protein degradation. Curr Opin Cell Biol 7: 215-223, 1995.
82. Hoffman-Goetz L, and Pedersen BK. Exercise and the immune system: a model of the stress response? Immunol Today 15: 382-387, 1994.
83. Holloszy JO. Adaptation of skeletal muscle to endurance exercise. Med Sci Sports 7: 155-164, 1975.
84. Holloszy JO. Biochemical Adaptations in Muscle. Effects of exercise on mitochondrial oxygen uptake and respiratory enzyme activity in skeletal muscle. J Biol Chem 242: 2278-2282, 1967.
85. Holloszy JO, and Booth FW. Biochemical adaptations to endurance exercise in muscle. Annu Rev Physiol 38: 273-291, 1976.
86. Holloszy JO, and Coyle EF. Adaptations of skeletal muscle to endurance exercise and their metabolic consequences. J Appl Physiol 56: 831-838, 1984.
87. Holloszy JO, Oscai LB, Don IJ, and Mole PA. Mitochondrial citric acid cycle and related enzymes: adaptive response to exercise. Biochem Biophys Res Commun 40: 1368-1373, 1970.
88. Hopper MK, Coggan AR, and Coyle EF. Exercise stroke volume relative to plasma-volume expansion. J Appl Physiol 64: 404-408, 1988.
89. Howarth KR, Moreau NA, Phillips SM, and Gibala MJ. Coingestion of protein with carbohydrate during recovery from endurance exercise stimulates skeletal muscle protein synthesis in humans. J Appl Physiol 106: 1394-1402, 2009.
90. Huang H, and Tindall DJ. Dynamic FoxO transcription factors. J Cell Sci 120: 2479-2487, 2007.
91. Irrcher I, Adhihetty PJ, Sheehan T, Joseph A-M, and Hood DA. PPARgamma coactivator-1alpha expression during thyroid hormone- and contractile activity-induced mitochondrial adaptations. Am J Physiol Cell Physiol 284: C1669-1677, 2003.
92. Ivy JL. Regulation of muscle glycogen repletion, muscle protein synthesis and repair following exercise. J Sports Sci and Med 3: 131-138, 2004.
93. Ivy JL, Ding Z, Hwang H, Cialdella-Kam LC, and Morrison PJ. Post exercise carbohydrate-protein supplementation: phosphorylation of muscle proteins involved in glycogen synthesis and protein translation. Amino Acids 35: 89-97, 2008.

94. Ivy JL, Goforth HW, Jr., Damon BM, McCauley TR, Parsons EC, and Price TB. Early postexercise muscle glycogen recovery is enhanced with a carbohydrate-protein supplement. J Appl Physiol 93: 1337-1344, 2002.
95. Ivy JL, and Holloszy JO. Persistent increase in glucose uptake by rat skeletal muscle following exercise. Am J Physiol Cell Physiol 241: C200-203, 1981.
96. Ivy JL, Katz AL, Cutler CL, Sherman WM, and Coyle EF. Muscle glycogen synthesis after exercise: effect of time of carbohydrate ingestion. J Appl Physiol 64: 1480-1485, 1988.
97. Ivy JL, and Kuo CH. Regulation of GLUT4 protein and glycogen synthase during muscle glycogen synthesis after exercise. Acta Physiologica Scandinavica 162: 295-304, 1998.
98. Ivy JL, Lee MC, Brozinick JT, Jr., and Reed MJ. Muscle glycogen storage after different amounts of carbohydrate ingestion. J Appl Physiol 65: 2018-2023, 1988.
99. Ivy JL, Miller W, Dover V, Goodyear LG, Sherman WM, Farrell S, and Williams H. Endurance improved by ingestion of a glucose polymer supplement. Med Sci Sports Exerc 15: 466-471, 1983.
100. Ivy JL, Res PT, Sprague RC, and Widzer MO. Effect of a carbohydrate-protein supplement on endurance performance during exercise of varying intensity. Int J Sport Nutr Exerc Metab 13: 382-395, 2003.
101. Jenkins JK, Malyak M, and Arend WP. The effects of interleukin-10 on interleukin-1 receptor antagonist and interleukin-1 beta production in human monocytes and neutrophils. Lymphokine Cytokine Res 13: 47-54, 1994.
102. Jentjens RL, Achten J, and Jeukendrup AE. High oxidation rates from combined carbohydrates ingested during exercise. Med Sci Sports Exerc 36: 1551-1558, 2004.
103. Jentjens RL, and Jeukendrup AE. High rates of exogenous carbohydrate oxidation from a mixture of glucose and fructose ingested during prolonged cycling exercise. Br J Nutr 93: 485-492, 2005.
104. Jentjens RLPG, Moseley L, Waring RH, Harding LK, and Jeukendrup AE. Oxidation of combined ingestion of glucose and fructose during exercise. J Appl Physiol 96: 1277-1284, 2004.
105. Jentjens RLPG, van Loon LJC, Mann CH, Wagenmakers AJM, and Jeukendrup AE. Addition of protein and amino acids to carbohydrates does not enhance postexercise muscle glycogen synthesis. J Appl Physiol 91: 839-846, 2001.
106. Jentjens RLPG, Venables MC, and Jeukendrup AE. Oxidation of exogenous glucose, sucrose, and maltose during prolonged cycling exercise. J Appl Physiol 96: 1285-1291, 2004.
107. Jeukendrup AE. Carbohydrate intake during exercise and performance. Nutrition 20: 669-677, 2004.
108. Jeukendrup AE, and Jentjens R. Oxidation of carbohydrate feedings during prolonged exercise: current thoughts, guidelines and directions for future research. Sports Med 29: 407-424, 2000.
109. Jeukendrup AE, Wagenmakers AJM, Stegen JHCH, Gijsen AP, Brouns F, and Saris WHM. Carbohydrate ingestion can completely suppress endogenous glucose production during exercise. Am J Physiol Endocrinol Metab 276: E672-683, 1999.

110. Kammer L, Ding Z, Wang B, Hara D, Liao Y-H, and Ivy J. Cereal and nonfat milk support muscle recovery following exercise. Journal of the International Society of Sports Nutrition 6: 11, 2009.
111. Karlsson HKR, Nilsson P-A, Nilsson J, Chibalin AV, Zierath JR, and Blomstrand E. Branched-chain amino acids increase p70S6k phosphorylation in human skeletal muscle after resistance exercise. Am J Physiol Endocrinol Metab 287: E1-7, 2004.
112. Kelly DP, and Scarpulla RC. Transcriptional regulatory circuits controlling mitochondrial biogenesis and function. Genes & Dev 18: 357-368, 2004.
113. Kim D-H, Sarbassov DD, Ali SM, King JE, Latek RR, Erdjument-Bromage H, Tempst P, and Sabatini DM. mTOR Interacts with Raptor to Form a Nutrient-Sensitive Complex that Signals to the Cell Growth Machinery. Cell 110: 163-175, 2002.
114. Kimball SR, Farrell PA, and Jefferson LS. Exercise Effects on Muscle Insulin Signaling and Action: Invited Review: Role of insulin in translational control of protein synthesis in skeletal muscle by amino acids or exercise. J Appl Physiol 93: 1168-1180, 2002.
115. Kimball SR, and Jefferson LS. Regulation of global and specific mRNA translation by oral administration of branched-chain amino acids. Biochem Biophys Res Commun 313: 423-427, 2004.
116. Kimball SR, and Jefferson LS. Signaling Pathways and Molecular Mechanisms through which Branched-Chain Amino Acids Mediate Translational Control of Protein Synthesis. J Nutr 136: 227S-231, 2006.
117. Konig D, Schumacher YO, Heinrich L, Schmid A, Berg A, and Dickhuth HH. Myocardial stress after competitive exercise in professional road cyclists. Med Sci Sports Exerc 35: 1679-1683, 2003.
118. Koopman R, Wagenmakers AJM, Manders RJF, Zorenc AHG, Senden JMG, Gorselink M, Keizer HA, and van Loon LJC. Combined ingestion of protein and free leucine with carbohydrate increases postexercise muscle protein synthesis in vivo in male subjects. Am J Physiol Endocrinol Metab 288: E645-653, 2005.
119. Kramer HF, and Goodyear LJ. Exercise, MAPK, and NF-{kappa}B signaling in skeletal muscle. J Appl Physiol 103: 388-395, 2007.
120. Kreider RB, Fry AC, and O'Toole ML editors. Overtraining in Sport. Champaign, IL: Human Kinetics, 1998.
121. Krzywkowski K, Petersen EW, Ostrowski K, Link-Amster H, Boza J, Halkjaer-Kristensen J, and Pedersen BK. Effect of glutamine and protein supplementation on exercise-induced decreases in salivary IgA. J Appl Physiol 91: 832-838, 2001.
122. Lancaster GI, Khan Q, Drysdale PT, Wallace F, Jeukendrup AE, Drayson MT, and Gleeson M. Effect of prolonged exercise and carbohydrate ingestion on type 1 and type 2 T lymphocyte distribution and intracellular cytokine production in humans. J Appl Physiol 98: 565-571, 2005.
123. Lawrie RA. Effect of enforced exercise on myoglobin concentration in muscle. Nature 171: 1069-1070, 1953.

124. Lecker SH, Goldberg AL, and Mitch WE. Protein Degradation by the Ubiquitin-Proteasome Pathway in Normal and Disease States. J Am Soc Nephrol 17: 1807-1819, 2006.
125. Leger B, Cartoni R, Praz M, Lamon S, Deriaz O, Crettenand A, Gobelet C, Rohmer P, Konzelmann M, Luthi F, and Russell AP. Akt signalling through GSK-3{beta}, mTOR and Foxo1 is involved in human skeletal muscle hypertrophy and atrophy. J Physiol 576: 923-933, 2006.
126. Leone TC, Lehman JJ, Finck BN, Schaeffer PJ, Wende AR, Boudina S, Courtois M, Wozniak DF, Sambandam N, Bernal-Mizrachi C, Chen Z, Holloszy JO, Medeiros DM, Schmidt RE, Saffitz JE, Abel ED, Semenkovich CF, and Kelly DP. PGC-1alpha deficiency causes multi-system energy metabolic derangements: muscle dysfunction, abnormal weight control and hepatic steatosis. PLoS Biol 3: e101, 2005.
127. Levenhagen DK, Carr C, Carlson MG, Maron DJ, Borel MJ, and Flakoll PJ. Postexercise protein intake enhances whole-body and leg protein accretion in humans. Med Sci Sports Exerc 34: 828-837, 2002.
128. Levenhagen DK, Gresham JD, Carlson MG, Maron DJ, Borel MJ, and Flakoll PJ. Postexercise nutrient intake timing in humans is critical to recovery of leg glucose and protein homeostasis. Am J Physiol Endocrinol Metab 280: E982-993, 2001.
129. Lin J, Handschin C, and Spiegelman BM. Metabolic control through the PGC-1 family of transcription coactivators. Cell Metab 1: 361-370, 2005.
130. Lin J, Wu H, Tarr PT, Zhang CY, Wu Z, Boss O, Michael LF, Puigserver P, Isotani E, Olson EN, Lowell BB, Bassel-Duby R, and Spiegelman BM. Transcriptional co-activator PGC-1 alpha drives the formation of slow-twitch muscle fibres. Nature 418: 797-801, 2002.
131. Lin YS, Jan MS, Tsai TJ, and Chen HI. Immunomodulatory effects of acute exercise bout in sedentary and trained rats. Med Sci Sports Exerc 27: 73-78, 1995.
132. Louis E, Raue U, Yang Y, Jemiolo B, and Trappe S. Time course of proteolytic, cytokine, and myostatin gene expression after acute exercise in human skeletal muscle. J Appl Physiol 103: 1744-1751, 2007.
133. Luden N, Saunders M, Pratt C, Bickford A, Todd M, and Flohr J. Effects of a Six-Day Carbohyydrate/Protein Intervention on Muscle Damage, Soreness, and Performance In Runners. Med Sci Sports Exc 38: S341, 2006.
134. Mackinnon LT. Immunity in Athletes Int J Sports Med 18: S62 - S68, 1997.
135. MacNeil B, Hoffman-Goetz L, Kendall A, Houston M, and Arumugam Y. Lymphocyte proliferation responses after exercise in men: fitness, intensity, and duration effects. J Appl Physiol 70: 179-185, 1991.
136. Mahan MP, and Young MR. Immune parameters of untrained or exercise-trained rats after exhaustive exercise. J Appl Physiol 66: 282-287, 1989.
137. Manolakos J, Tang J, Kujbida G, Lysecki P, Moore D, and Phillips S. Minimal whey protein with carbohydrate stimulates muscle protein synthesis following resistance exercise in young men. FASEB J 21: LB108-a-, 2007.

138. Margaritis I, Tessier F, Richard MJ, and Marconnet P. No evidence of oxidative stress after a triathlon race in highly trained competitors. Int J Sports Med 18: 186-190, 1997.
139. Martin DE, and Hall MN. The expanding TOR signaling network. Current Opinion in Cell Biology 17: 158-166, 2005.
140. Martinez-Lagunas V, Ding Z, Bernard JR, Wang B, and Ivy JL. Added Protein Maintains Efficacy of a Low-Carbohydrate Sports Drink. J Strength Cond Res 2009.
141. Mascher H, Andersson H, Nilsson PA, Ekblom B, and Blomstrand E. Changes in signalling pathways regulating protein synthesis in human muscle in the recovery period after endurance exercise. Acta Physiol (Oxf) 191: 67-75, 2007.
142. McCarthy DA, and Dale MM. The leucocytosis of exercise. A review and model. Sports Med 6: 333-363, 1988.
143. Miller SL, Tipton KD, Chinkes DL, Wolf SE, and Wolfe RR. Independent and combined effects of amino acids and glucose after resistance exercise. Med Sci Sports Exerc 35: 449-455, 2003.
144. Moldoveanu AI, Shephard RJ, and Shek PN. Exercise elevates plasma levels but not gene expression of IL-1beta , IL-6, and TNF-alpha in blood mononuclear cells. J Appl Physiol 89: 1499-1504, 2000.
145. Morrison PJ, Hara D, Ding Z, and Ivy JL. Adding protein to a carbohydrate supplement provided after endurance exercise enhances 4E-BP1 and RPS6 signaling in skeletal muscle. J Appl Physiol 104: 1029-1036, 2008.
146. Morton JP, Croft L, Bartlett JD, MacLaren DPM, Reilly T, Evans L, McArdle A, and Drust B. Reduced carbohydrate availability does not modulate training-induced heat shock protein adaptations but does upregulate oxidative enzyme activity in human skeletal muscle. J Appl Physiol 106: 1513-1521, 2009.
147. Mougios V. Reference intervals for serum creatine kinase in athletes. Br J Sports Med 41: 674-678, 2007.
148. Nakae J, Oki M, and Cao Y. The FoxO transcription factors and metabolic regulation. FEBS Lett 582: 54-67, 2008.
149. Nave BT, Ouwens M, Withers DJ, Alessi DR, and Shepherd PR. Mammalian target of rapamycin is a direct target for protein kinase B: identification of a convergence point for opposing effects of insulin and amino-acid deficiency on protein translation. Biochem J 344 Pt 2: 427-431, 1999.
150. Nehlsen-Cannarella SL, Fagoaga OR, Nieman DC, Henson DA, Butterworth DE, Schmitt RL, Bailey EM, Warren BJ, Utter A, and Davis JM. Carbohydrate and the cytokine response to 2.5 h of running. J Appl Physiol 82: 1662-1667, 1997.
151. Newham DJ, Jones DA, and Edwards RH. Large delayed plasma creatine kinase changes after stepping exercise. Muscle Nerve 6: 380-385, 1983.
152. Nielson CP. Beta-adrenergic modulation of the polymorphonuclear leukocyte respiratory burst is dependent upon the mechanism of cell activation. J Immunol 139: 2392-2397, 1987.
153. Nieman DC. Exercise, infection, and immunity. Int J Sports Med 15 Suppl 3: S131-141, 1994.

154. Nieman DC. Immune response to heavy exertion. J Appl Physiol 82: 1385-1394, 1997.
155. Nieman DC. Influence of carbohydrate on the immune response to intensive, prolonged exercise. Exerc Immunol Rev 4: 64-76, 1998.
156. Nieman DC. Special Feature for the Olympics: Effects of Excercise on the Immune System. Immunology and Cell Biology 78: 496-501, 2000.
157. Nieman DC, Berk LS, Simpson-Westerber M, Arabatzis K, Youngberg S, Tan SA, Lee JW, and Eby WC. Effects of long-endurance running on immune system parameters and lymphocyte function in experienced marathoners. Int J Sports Med 10: 317-323, 1989.
158. Nieman DC, and Bishop NC. Nutritional strategies to counter stress to the immune system in athletes, with special reference to football. J Sports Sci 24: 763-772, 2006.
159. Nieman DC, Davis JM, Henson DA, Walberg-Rankin J, Shute M, Dumke CL, Utter AC, Vinci DM, Carson JA, Brown A, Lee WJ, McAnulty SR, and McAnulty LS. Carbohydrate ingestion influences skeletal muscle cytokine mRNA and plasma cytokine levels after a 3-h run. J Appl Physiol 94: 1917-1925, 2003.
160. Nieman DC, Fagoaga OR, Butterworth DE, Warren BJ, Utter A, Davis JM, Henson DA, and Nehlsen-Cannarella SL. Carbohydrate supplementation affects blood granulocyte and monocyte trafficking but not function after 2.5 h or running. Am J Clinical Nutrition 66: 153-159, 1997.
161. Nieman DC, Henson DA, Austin MD, and Brown VA. Immune response to a 30-minute walk. Med Sci Sports Exerc 37: 57-62, 2005.
162. Nieman DC, Henson DA, Davis JM, Angela Murphy E, Jenkins DP, Gross SJ, Carmichael MD, Quindry JC, Dumke CL, Utter AC, McAnulty SR, McAnulty LS, Triplett NT, and Mayer EP. Quercetin's influence on exercise-induced changes in plasma cytokines and muscle and leukocyte cytokine mRNA. J Appl Physiol 103: 1728-1735, 2007.
163. Nieman DC, Henson DA, Davis JM, Dumke CL, Utter AC, Murphy EA, Pearce S, Gojanovich G, McAnulty SR, and McAnulty LS. Blood leukocyte mRNA expression for IL-10, IL-1Ra, and IL-8, but not IL-6, increases after exercise. J Interferon Cytokine Res 26: 668-674, 2006.
164. Nieman DC, Henson DA, Gross SJ, Jenkins DP, Davis JM, Murphy AE, Carmichael MD, Dumke CL, Utter AC, McAnulty SR, McAnulty LS, and Mayer EP. Quercetin Reduces Illness but Not Immune Perturbations after Intensive Exercise. . Med Sci Sports Exc 39: 1561-1569, 2007.
165. Nieman DC, Henson DA, McAnulty SR, McAnulty LS, Morrow JD, Ahmed A, and Heward CB. Vitamin E and immunity after the Kona Triathlon World Championship. Med Sci Sports Exc 36: 1328-1335, 2004.
166. Nieman DC, Henson DA, Smith LL, Utter AC, Vinci DM, Davis JM, Kaminsky DE, and Shute M. Cytokine changes after a marathon race. J Appl Physiol 91: 109-114, 2001.
167. Nieman DC, Johanssen LM, Lee JW, and Arabatzis K. Infectious episodes in runners before and after the Los Angeles Marathon. J Sports Med Phys Fitness 30: 316-328, 1990.

168. Nieman DC, Miller AR, Henson DA, Warren BJ, Gusewitch G, Johnson RL, Davis JM, Butterworth DE, Herring JL, and Nehlsen-Cannarell SL. Effect of high- versus moderate-intensity exercise on lymphocyte subpopulations and proliferative response. Int J Sports Med 15: 199-206, 1994.
169. Nieman DC, Nehlsen-Cannarell SL, Fagoaga OR, Henson DA, Utter A, Davis JM, Williams F, and Butterworth DE. Influence of mode and carbohydrate on the cytokine response to heavy exertion. Med Sci Sports Exerc 30: 671-678, 1998.
170. Nieman DC, and Nehlsen-Cannarella SL. The effects of acute and chronic exercise of immunoglobulins. Sports Med 11: 183-201, 1991.
171. Nieman DC, Nehlsen-Cannarella SL, Fagoaga OR, Henson DA, Utter A, Davis JM, Williams F, and Butterworth DE. Effects of mode and carbohydrate on the granulocyte and monocyte response to intensive, prolonged exercise. J Appl Physiol 84: 1252-1259, 1998.
172. Nieman DC, and Pedersen BK. Exercise and immune function. Recent developments. Sports Med 27: 73-80, 1999.
173. Niles ES, Lachowetz T, Garfi J, Sullivan W, Smith JC, Leyh BP, and Headley SA. Carbohydrate-Protein Drink Improves Time to Exhaustion after Recovery from Endurance Exercise. JEPonline 4: 45-52, 2001.
174. Okazaki K, Ichinose T, Mitono H, Chen M, Masuki S, Endoh H, Hayase H, Doi T, and Nose H. Impact of protein and carbohydrate supplementation on plasma volume expansion and thermoregulatory adaptation by aerobic training in older men. J Appl Physiol 107: 725-733, 2009.
175. O'Reilly KP, Warhol MJ, Fielding RA, Frontera WR, Meredith CN, and Evans WJ. Eccentric exercise-induced muscle damage impairs muscle glycogen repletion. J Appl Physiol 63: 252-256, 1987.
176. Osterberg KL, Zachwieja JJ, and Smith JW. Carbohydrate and carbohydrate+protein for cycling time-trial performance. Journal of Sports Sciences 26: 227 - 233, 2008.
177. Ostrowski K, Hermann C, Bangash A, Schjerling P, Nielsen JN, and Pedersen BK. A trauma-like elevation of plasma cytokines in humans in response to treadmill running. J Physiol 513: 889-894, 1998.
178. Ostrowski K, Rohde T, Asp S, Schjerling P, and Pedersen BK. Pro- and anti-inflammatory cytokine balance in strenuous exercise in humans. J Physiol 515: 287-291, 1999.
179. Ostrowski K, Rohde T, Zacho M, Asp S, and Pedersen BK. Evidence that interleukin-6 is produced in human skeletal muscle during prolonged running. J Physiol 508: 949-953, 1998.
180. Parry-Billings M, Budgett R, Koutedakis Y, Blomstrand E, Brooks S, Williams C, Calder PC, Pilling S, Baigrie R, and Newsholme EA. Plasma amino acid concentrations in the overtraining syndrome: possible effects on the immune system. Med Sci Sports Exerc 24: 1353-1358, 1992.
181. Pattengale PK, and Holloszy JO. Augmentation of skeletal muscle myoglobin by a program of treadmill running. Am J Physiol -- Legacy Content 213: 783-785, 1967.
182. Patti M-E, Brambilla E, Luzi L, Landaker EJ, and Ronald Kahn C. Bidirectional Modulation of Insulin Action by Amino Acids. J Clin Invest 101: 1519-1529, 1998.

183. Pedersen BK, Akerstrom TCA, Nielsen AR, and Fischer CP. Role of myokines in exercise and metabolism. J Appl Physiol 103: 1093-1098, 2007.
184. Pedersen BK, Bruunsgaard H, Klokker M, Kappel M, MacLean DA, Nielsen HB, Rohde T, Ullum H, and Zacho M. Exercise-induced immunomodulation--possible roles of neuroendocrine and metabolic factors. Int J Sports Med 18 Suppl 1: S2-7, 1997.
185. Pedersen BK, and Hoffman-Goetz L. Exercise and the Immune System: Regulation, Integration, and Adaptation. Physiol Rev 80: 1055-1081, 2000.
186. Pedersen BK, and Nieman DC. Exercise immunology: integration and regulation. Immunology Today 19: 204-206, 1998.
187. Pedersen BK, Rohde T, and Zacho M. Immunity in athletes. J Sports Med Phys Fitness 36: 236-245, 1996.
188. Pedersen BK, Steensberg A, and Schjerling P. Muscle-derived interleukin-6: possible biological effects. J Physiol 536: 329-337, 2001.
189. Pedersen BK, and Toft AD. Effects of exercise on lymphocytes and cytokines. Br J Sports Med 34: 246-251, 2000.
190. Pedersen BK, Tvede N, Christensen LD, Klarlund K, Kragbak S, and Halkjr-Kristensen J. Natural killer cell activity in peripheral blood of highly trained and untrained persons. Int J Sports Med 10: 129-131, 1989.
191. Pende M, Um SH, Mieulet V, Sticker M, Goss VL, Mestan J, Mueller M, Fumagalli S, Kozma SC, and Thomas G. S6K1-/-/S6K2-/- Mice Exhibit Perinatal Lethality and Rapamycin-Sensitive 5'-Terminal Oligopyrimidine mRNA Translation and Reveal a Mitogen-Activated Protein Kinase-Dependent S6 Kinase Pathway. Mol Cell Biol 24: 3112-3124, 2004.
192. Petersen AMW, and Pedersen BK. The anti-inflammatory effect of exercise. J Appl Physiol 98: 1154-1162, 2005.
193. Petersen EW, Carey AL, Sacchetti M, Steinberg GR, Macaulay SL, Febbraio MA, and Pedersen BK. Acute IL-6 treatment increases fatty acid turnover in elderly humans in vivo and in tissue culture in vitro. Am J Physiol Endocrinol Metab 288: E155-162, 2005.
194. Phillips SM, Green HJ, Tarnopolsky MA, Heigenhauser GJ, and Grant SM. Progressive effect of endurance training on metabolic adaptations in working skeletal muscle. Am J Physiol Endocrinol Metab 270: E265-272, 1996.
195. Phillips SM, Tipton KD, Aarsland A, Wolf SE, and Wolfe RR. Mixed muscle protein synthesis and breakdown after resistance exercise in humans. Am J Physiol Endocrinol Metab 273: E99-107, 1997.
196. Piehl-Aulin K, Söderlund K, and Hultman E. Muscle glycogen resynthesis rate in humans after supplementation of drinks containing carbohydrates with low and high molecular masses European Journal of Applied Physiology 81: 346-351, 2000.
197. Pierce AP, de Waal E, McManus LM, Shireman PK, and Chaudhuri AR. Oxidation and structural perturbation of redox-sensitive enzymes in injured skeletal muscle. Free Radic Biol Med 43: 1584-1593, 2007.
198. Powers S, and Howley E. Exercise Physiology: Theory and Application to Fitness and Performance. New York, NY: McGraw-Hill, 2004.

199. Pratt C, Saunders M, Moore R, Hammer M, Todd M, Flohr J, and Kies A. Improved Endurance Performance With Carbohydrate- Protein Hydrolysate Beverage Not Due To Attenuated Central Fatigue. Med Sci Sports Exc 39: S362, 2007.
200. Pritchett K, Bishop P, Pritchett R, Green M, and Katica C. Acute effects of chocolate milk and a commercial recovery beverage on postexercise recovery indices and endurance cycling performance. Appl Physiol Nutr Metab 34: 1017-1022, 2009.
201. Proud CG. Regulation of mammalian translation factors by nutrients. Eur J Biochem 269: 5338-5349, 2002.
202. Puigserver P, and Spiegelman BM. Peroxisome Proliferator-Activated Receptor-{gamma} Coactivator 1{alpha} (PGC-1{alpha}): Transcriptional Coactivator and Metabolic Regulator. Endocr Rev 24: 78-90, 2003.
203. Puntschart A, Claassen H, Jostarndt K, Hoppeler H, and Billeter R. mRNAs of enzymes involved in energy metabolism and mtDNA are increased in endurance-trained athletes. Am J Physiol Cell Physiol 269: C619-625, 1995.
204. Rasmussen BB, Tipton KD, Miller SL, Wolf SE, and Wolfe RR. An oral essential amino acid-carbohydrate supplement enhances muscle protein anabolism after resistance exercise. J Appl Physiol 88: 386-392, 2000.
205. Raue U, Slivka D, Jemiolo B, Hollon C, and Trappe S. Proteolytic Gene Expression Differs At Rest and After Resistance Exercise Between Young and Old Women. J Gerontol A Biol Sci Med Sci 62: 1407-1412, 2007.
206. Reed MJ, Brozinick JT, Jr., Lee MC, and Ivy JL. Muscle glycogen storage postexercise: effect of mode of carbohydrate administration. J Appl Physiol 66: 720-726, 1989.
207. Riedy M, Moore RL, and Gollnick PD. Adaptive response of hypertrophied skeletal muscle to endurance training. J Appl Physiol 59: 127-131, 1985.
208. Rohde T, MacLean DA, Hartkopp A, and Pedersen BK. The immune system and serum glutamine during a triathlon. Eur J Appl Physiol Occup Physiol 74: 428-434, 1996.
209. Romano B, Todd M, and Saunders M. Effect of a 4: 1 Ratio Carbohydrate/Protein Beverage on Endurance Performance, Muscle Damage and Recovery. Med Sci Sports Exc 36: S126, 2004.
210. Romano-Ely B, Todd M, Saunders M, and St. Laurent T. Effect of an Isocaloric Carbohydrate-Protein-Antioxidant Drink on Cycling Performance. Med Sci Sports Exc 38: 1608-1616, 2006.
211. Roux PP, and Blenis J. ERK and p38 MAPK-Activated Protein Kinases: a Family of Protein Kinases with Diverse Biological Functions. Microbiol Mol Biol Rev 68: 320-344, 2004.
212. Rowlands DS, Thorp RM, Rossler K, Graham DF, and Rockell MJ. Effect of protein-rich feeding on recovery after intense exercise. Int J Sport Nutr Exerc Metab 17: 521-543, 2007.
213. Roy BD, Tarnopolsky MA, Macdougall JD, Fowles J, and Yarasheski KE. Effect of glucose supplement timing on protein metabolism after resistance training. J Appl Physiol 82: 1882-1888, 1997.

214. Saltin B, Blomqvist G, JH Mitchell J, Johnson Jr R, Wildenthal K, and Chapman C. A Longitudinal Study of Adaptive Changes in Oxygen Transport and Body Composition. Circulation 38: VII 1-78, 1968.
215. Sarbassov DD, Guertin DA, Ali SM, and Sabatini DM. Phosphorylation and Regulation of Akt/PKB by the Rictor-mTOR Complex. Science 307: 1098-1101, 2005.
216. Sarbassov DD, and Sabatini DM. Redox Regulation of the Nutrient-sensitive Raptor-mTOR Pathway and Complex. J Biol Chem 280: 39505-39509, 2005.
217. Saunders M, Kane M, and Todd K. Effects of a Carbohydrate-Protein Beverage on Cycling Endurance and Muscle Damage. Med Sci Sports Exc 36: 1233-1238, 2004.
218. Saunders M, Todd M, Valentine R, St. Laurent T, Kane M, Luden N, and Herrick J. Inter-Study Examination of Physiological Variables Associated with Improved Endurance Performance with Carbohydrate/Protein Administration. Med Sci Sports Exc 38: S113-S114, 2006.
219. Saunders MJ, Luden ND, and Herrick JE. Consumption of an oral carbohydrate-protein gel improves cycling endurance and prevents postexercise muscle damage. J Strength Cond Res 21: 678-684, 2007.
220. Scharhag J, Meyer T, Auracher M, Gabriel HH, and Kindermann W. Effects of graded carbohydrate supplementation on the immune response in cycling. Med Sci Sports Exerc 38: 286-292, 2006.
221. Scharhag J, Meyer T, Auracher M, Muller M, Herrmann M, Gabriel H, Herrmann W, and Kindermann W. Exercise-induced increases in NT-proBNP are not related to the exercise-induced immune response. Br J Sports Med 42: 383-385, 2008.
222. Scholander PF. Oxygen Transport through Hemoglobin Solutions. Science 131: 585-590, 1960.
223. Selkirk GA, McLellan TM, Wright HE, and Rhind SG. Mild endotoxemia, NF-{kappa}B translocation, and cytokine increase during exertional heat stress in trained and untrained individuals. Am J Physiol Regulatory Integrative Comp Physiol 295: R611-623, 2008.
224. Shing CM, Peake J, Suzuki K, Okutsu M, Pereira R, Stevenson L, Jenkins DG, and Coombes JS. Effects of bovine colostrum supplementation on immune variables in highly trained cyclists. J Appl Physiol 102: 1113-1122, 2007.
225. Smith LL. Cytokine hypothesis of overtraining: a physiological adaptation to excessive stress? Med Sci Sports Exerc 32: 317-331, 2000.
226. Sorichter S, Mair J, Koller A, Gebert W, Rama D, Calzolari C, Artner-Dworzak E, and Puschendorf B. Skeletal troponin I as a marker of exercise-induced muscle damage. J Appl Physiol 83: 1076-1082, 1997.
227. Spiller GA, Jensen CD, Pattison TS, Chuck CS, Whittam JH, and Scala J. Effect of protein dose on serum glucose and insulin response to sugars. Am J Clinical Nutrition 46: 474-480, 1987.
228. Spina RJ, Chi MM, Hopkins MG, Nemeth PM, Lowry OH, and Holloszy JO. Mitochondrial enzymes increase in muscle in response to 7-10 days of cycle exercise. J Appl Physiol 80: 2250-2254, 1996.

229. Sprenger H, Jacobs C, Nain M, Gressner AM, Prinz H, Wesemann W, and Gemsa D. Enhanced release of cytokines, interleukin-2 receptors, and neopterin after long-distance running. Clin Immunol Immunopathol 63: 188-195, 1992.
230. Stitt TN, Drujan D, Clarke BA, Panaro F, Timofeyva Y, Kline WO, Gonzalez M, Yancopoulos GD, and Glass DJ. The IGF-1/PI3K/Akt pathway prevents expression of muscle atrophy-induced ubiquitin ligases by inhibiting FOXO transcription factors. Mol Cell 14: 395-403, 2004.
231. Suzuki K, Naganuma S, Totsuka M, Suzuki KJ, Mochizuki M, Shiraishi M, Nakaji S, and Sugawara K. Effects of exhaustive endurance exercise and its one-week daily repetition on neutrophil count and functional status in untrained men. Int J Sports Med 17: 205-212, 1996.
232. Suzuki K, Nakaji S, Yamada M, Totsuka M, Sato K, and Sugawara K. Systemic inflammatory response to exhaustive exercise. Cytokine kinetics. Exerc Immunol Rev 8: 6-48, 2002.
233. Suzuki K, Sato H, Kikuchi T, Abe T, Nakaji S, Sugawara K, Totsuka M, Sato K, and Yamaya K. Capacity of circulating neutrophils to produce reactive oxygen species after exhaustive exercise. J Appl Physiol 81: 1213-1222, 1996.
234. Suzuki K, Totsuka M, Nakaji S, Yamada M, Kudoh S, Liu Q, Sugawara K, Yamaya K, and Sato K. Endurance exercise causes interaction among stress hormones, cytokines, neutrophil dynamics, and muscle damage. J Appl Physiol 87: 1360-1367, 1999.
235. Taillandier D, Aurousseau E, Meynial-Denis D, Bechet D, Ferrara M, Cottin P, Ducastaing A, Bigard X, Guezennec CY, and Schmid HP. Coordinate activation of lysosomal, Ca 2+-activated and ATP-ubiquitin-dependent proteinases in the unweighted rat soleus muscle. Biochem J 316 (Pt 1): 65-72, 1996.
236. Tarnopolsky MA, Bosman M, Macdonald JR, Vandeputte D, Martin J, and Roy BD. Postexercise protein-carbohydrate and carbohydrate supplements increase muscle glycogen in men and women. J Appl Physiol 83: 1877-1883, 1997.
237. Terada S, Goto M, Kato M, Kawanaka K, Shimokawa T, and Tabata I. Effects of low-intensity prolonged exercise on PGC-1 mRNA expression in rat epitrochlearis muscle. Biochem Biophys Res Commun 296: 350-354, 2002.
238. Thomas K, Morris P, and Stevenson E. Improved endurance capacity following chocolate milk consumption compared with 2 commercially available sport drinks. Appl Physiol Nutr Metab 34: 78-82, 2009.
239. Tipton KD, Elliott TA, Cree MG, Wolf SE, Sanford AP, and Wolfe RR. Ingestion of casein and whey proteins result in muscle anabolism after resistance exercise. Med Sci Sports Exerc 36: 2073-2081, 2004.
240. Tipton KD, Ferrando AA, Phillips SM, Doyle D, Jr., and Wolfe RR. Postexercise net protein synthesis in human muscle from orally administered amino acids. Am J Physiol Endocrinol Metab 276: E628-634, 1999.
241. Toft AD, Jensen LB, Bruunsgaard H, Ibfelt T, Halkjar-Kristensen J, Febbraio M, and Pedersen BK. Cytokine response to eccentric exercise in young and elderly humans. Am J Physiol Cell Physiol 283: C289-295, 2002.

242. Totsuka M, Naganuma S, Suzuki K, Nakaji S, Sato S, and Sugawara S. Rapid adaptation of creatine kinase responses to repeated daily endurance exercise. J Phys Fitness Nutr Immunol 6: 187-190, 1996.
243. Totsuka M, Nakaji S, Suzuki K, Sugawara K, and Sato K. Break point of serum creatine kinase release after endurance exercise. J Appl Physiol 93: 1280-1286, 2002.
244. Ueki K, Yamamoto-Honda R, Kaburagi Y, Yamauchi T, Tobe K, Burgering BMT, Coffer PJ, Komuro I, Akanuma Y, Yazaki Y, and Kadowaki T. Potential Role of Protein Kinase B in Insulin-induced Glucose Transport, Glycogen Synthesis, and Protein Synthesis. J Biol Chem 273: 5315-5322, 1998.
245. Ullum H, Haahr PM, Diamant M, Palmo J, Halkjaer-Kristensen J, and Pedersen BK. Bicycle exercise enhances plasma IL-6 but does not change IL-1 alpha, IL-1 beta, IL-6, or TNF-alpha pre-mRNA in BMNC. J Appl Physiol 77: 93-97, 1994.
246. Valentine RJ, Saunders MJ, Todd MK, and St Laurent TG. Influence of carbohydrate-protein beverage on cycling endurance and indices of muscle disruption. Int J Sport Nutr Exerc Metab 18: 363-378, 2008.
247. van Essen M, and Gibala MJ. Failure of protein to improve time trial performance when added to a sports drink. Med Sci Sports Exerc 38: 1476-1483, 2006.
248. van Hall G, Shirreffs SM, and Calbet JAL. Muscle glycogen resynthesis during recovery from cycle exercise: no effect of additional protein ingestion. J Appl Physiol 88: 1631-1636, 2000.
249. van Hall G, Steensberg A, Sacchetti M, Fischer C, Keller C, Schjerling P, Hiscock N, Moller K, Saltin B, Febbraio MA, and Pedersen BK. Interleukin-6 Stimulates Lipolysis and Fat Oxidation in Humans. J Clin Endocrinol Metab 88: 3005-3010, 2003.
250. van Loon LJC, Saris WHM, Kruijshoop M, and Wagenmakers AJM. Maximizing postexercise muscle glycogen synthesis: carbohydrate supplementation and the application of amino acid or protein hydrolysate mixtures. Am J Clinical Nutrition 72: 106-111, 2000.
251. Wagenmakers AJ, Brouns F, Saris WH, and Halliday D. Oxidation rates of orally ingested carbohydrates during prolonged exercise in men. J Appl Physiol 75: 2774-2780, 1993.
252. Walsh NP, Blannin AK, Bishop NC, Robson PJ, and Gleeson M. Effect of oral glutamine supplementation on human neutrophil lipopolysaccharide-stimulated degranulation following prolonged exercise. Int J Sport Nutr Exerc Metab 10: 39-50, 2000.
253. Walsh NP, Blannin AK, Robson PJ, and Gleeson M. Glutamine, exercise and immune function. Links and possible mechanisms. Sports Med 26: 177-191, 1998.
254. Wang L, Rhodes CJ, and Lawrence JC, Jr. Activation of Mammalian Target of Rapamycin (mTOR) by Insulin Is Associated with Stimulation of 4EBP1 Binding to Dimeric mTOR Complex 1. J Biol Chem 281: 24293-24303, 2006.
255. Wang X, and Proud CG. A Novel Mechanism for the Control of Translation Initiation by Amino Acids, Mediated by Phosphorylation of Eukaryotic Initiation Factor 2B. Mol Cell Biol 28: 1429-1442, 2008.

256. Wilkinson SB, Tarnopolsky MA, MacDonald MJ, MacDonald JR, Armstrong D, and Phillips SM. Consumption of fluid skim milk promotes greater muscle protein accretion after resistance exercise than does consumption of an isonitrogenous and isoenergetic soy-protein beverage. Am J Clinical Nutrition 85: 1031-1040, 2007.
257. Williams MB, Raven PB, Fogt DL, and Ivy JL. Effects of Recovery Beverages on Glycogen Restoration and Endurance Exercise Performance. Journal of Strength and Conditioning Research 17: 12-19, 2003.
258. Williamson DL, Kubica N, Kimball SR, and Jefferson LS. Exercise-induced alterations in extracellular signal-regulated kinase 1/2 and mammalian target of rapamycin (mTOR) signalling to regulatory mechanisms of mRNA translation in mouse muscle. J Physiol 573: 497-510, 2006.
259. Williamson DL, Raue U, Slivka DR, and Trappe S. Resistance Exercise, Skeletal Muscle FOXO3A, and 85-Year-Old Women. J Gerontol A Biol Sci Med Sci 65A: 335-343, 2010.
260. Wojcik JR, Walber-Rankin J, Smith LL, and Gwazdauskas FC. Comparison of carbohydrate and milk-based beverages on muscle damage and glycogen following exercise. Int J Sport Nutr Exerc Metab 11: 406-419, 2001.
261. Wright DC, Han D-H, Garcia-Roves PM, Geiger PC, Jones TE, and Holloszy JO. Exercise-induced Mitochondrial Biogenesis Begins before the Increase in Muscle PGC-1{alpha} Expression. J Biol Chem 282: 194-199, 2007.
262. Wu Z, Puigserver P, Andersson U, Zhang C, Adelmant G, Mootha V, Troy A, Cinti S, Lowell B, Scarpulla RC, and Spiegelman BM. Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. Cell 98: 115-124, 1999.
263. Yaspelkis BB, 3rd, and Ivy JL. Effect of carbohydrate supplements and water on exercise metabolism in the heat. J Appl Physiol 71: 680-687, 1991.
264. Yaspelkis BB, 3rd, Patterson JG, Anderla PA, Ding Z, and Ivy JL. Carbohydrate supplementation spares muscle glycogen during variable-intensity exercise. J Appl Physiol 75: 1477-1485, 1993.
265. Zaldivar F, Wang-Rodriguez J, Nemet D, Schwindt C, Galassetti P, Mills PJ, Wilson LD, and Cooper DM. Constitutive pro- and anti-inflammatory cytokine and growth factor response to exercise in leukocytes. J Appl Physiol 100: 1124-1133, 2006.
266. Zawadzki KM, Yaspelkis BB, 3rd, and Ivy JL. Carbohydrate-protein complex increases the rate of muscle glycogen storage after exercise. J Appl Physiol 72: 1854-1859, 1992.

Chapter III: The Effect of a Low Carbohydrate Beverage with Added Protein on Cycling Endurance Performance in Trained Athletes

ABSTRACT

Ingesting carbohydrate plus protein during prolonged variable intensity exercise has demonstrated improved aerobic endurance performance beyond that of a carbohydrate supplement alone. The purpose of the present study was to determine if a supplement containing a mixture of different carbohydrates (glucose, maltodextrin and fructose) and a moderate amount of protein given during endurance exercise would increase time to exhaustion, despite containing 50% less total carbohydrate than a carbohydrate-only supplement. We also sought *post priori* to determine if there was a difference in effect based on percentage of ventilatory threshold at which the subjects cycled to exhaustion. Fifteen trained male and female cyclists exercised on two separate occasions at intensities alternating between 45% and 70% VO_2max for 3 h, after which the work load increased to ~74-85% VO_2max until exhaustion. Supplements (275 ml) were provided every 20 min during exercise and consisted of a 3% carbohydrate/1.2% protein supplement (MCP) and a 6% carbohydrate supplement (CHO). For the combined group (n=15), time to exhaustion (TTE) in MCP did not differ from CHO (31.06 ± 5.76 vs 26.03 ± 4.27 min, respectively, p=0.064). However, for subjects cycling at or below VT (n=8), TTE in MCP was significantly greater than CHO (45.64 ± 7.38 vs 35.47 ± 5.94 min, respectively, p=0.006). There were no significant differences in TTE for the above VT group (n=7). Our results suggest that, compared to a traditional 6% CHO supplement, a mixture of carbohydrates plus a moderate amount of protein can improve aerobic endurance at exercise intensities near the VT, despite containing lower total carbohydrate and caloric content.

INTRODUCTION

It has long been recognized that endurance exercise performance is significantly improved when carbohydrate is ingested during exercise compared to water only or placebo beverages (5,6,14,34,35). Several recent investigations have reported significant improvements in endurance exercise performance when a carbohydrate-protein (CHO+PRO) beverage is ingested during exercise compared to a carbohydrate-only (CHO) beverage (15,27,28). The added amount of protein is typically 25 to 30% of the total energy content of the beverage, yet appears to produce performance benefits beyond that of traditional carbohydrate-only beverages (3,15,25,27,28,33), as well as reduce muscle damage (2,27,28). However, many athletes and recreational exercisers desire a lower carbohydrate, lower caloric content alternative when maintaining or reducing body weight is a goal, in addition to improving fitness and endurance.

Previous work in our laboratory demonstrated that a lower CHO beverage with added protein was as effective in extending time to exhaustion as higher carbohydrate and higher calorie beverages (24). Martinez-Lagunas et al. (24) recently compared the effects of a 4.5% CHO plus 1.15% PRO, and a 3% CHO plus 0.75% PRO beverage, to a traditional 6% CHO beverage and found that there was no difference in the times to exhaustion between the treatments. This suggests that the efficacy of the supplements was maintained despite the reduction in total CHO and total energy content with the substitution of a small amount of protein (24). Based on these findings, we sought to determine if a lower CHO, lower calorie beverage containing a moderate amount of protein could be optimized using a mixture of CHO sources (glucose [dextrose], maltodextrin and fructose) rather than a single CHO (dextrose). Previous investigations have shown that maximal rate of exogenous carbohydrate utilization is about 1.0-1.1 g/min when a single carbohydrate source (e.g., dextrose) is ingested (21,22,23,31), but when multiple carbohydrate types (e. g., dextrose and fructose) are ingested, this rate can be increased significantly (17,19-21). Furthermore, Currell and colleagues (7) demonstrated an 8% improvement in time to complete a subsequent time trial when a mixture of glucose and fructose was ingested during a previous bout of prolonged

exercise compared to glucose only. The use of different stereospecific intestinal carbohydrate transporters for the different carbohydrates used is most likely the reason for the improved exogenous carbohydrate oxidation and thus, improved performance.

Therefore, the purpose of the present study was (1) to assess the efficacy of a supplement containing a mixture of carbohydrates (dextrose, maltodextrin, and fructose), yet a lower total carbohydrate content, and a moderate amount of protein (MCP, 3.0% CHO, 1.2% PRO), in extending time to exhaustion, and (2) to determine if muscle damage would be reduced by the protein-containing supplement. We hypothesized that time to exhaustion would be extended and muscle damage reduced with the MCP supplement compared to CHO, despite the MCP supplement containing 50% less carbohydrate and 30% fewer calories.

METHODS

Experimental Approach to the Problem

After first determining each subject's VO_2max, each subject completed a familiarization trial in which only water was provided, followed by 2 double-blinded, randomly-ordered experimental trials in which a 6% carbohydrate beverage (CHO) or a 3% carbohydrate/1.2% protein (MCP) beverage was provided during exercise. The CHO beverage consisted of dextrose, and the MCP beverage contained dextrose, maltodextrin, and fructose (1% each), and a whey protein isolate. At the beginning of exercise and every 20 min thereafter, 275 ml of the selected beverage (CHO or MCP) was consumed. The subjects performed each trial in a room of ~21° C at the same time of the day and the same day of the week over a 3-week period. Human Performance Laboratories, LLC (Austin, TX) provided the beverages in powder form, and were mixed in the laboratory to the concentrations specified above. The energy and macronutrient content of the beverages are shown in Table 3.1. The beverages were similar in color, taste, and texture

to allow a double-blinded and randomly ordered study design. A laboratory technician who was not involved in the data collection prepared the beverages for each trial.

The cycling protocol for all rides is shown in Figure 3.1. Subjects rode the cycle ergometer at intensities alternating between 45% and 70% VO_2max for 3 h, and then the workload was increased to ~74-85% VO_2max until exhaustion. The outcome measures of interest were: time to exhaustion (TTE), substrate utilization, and responses of insulin, glucose, lactate, and myoglobin to the different treatments.

Subjects

Fifteen trained endurance athletes (cyclists and triathletes) between the ages of 20 and 40 years were admitted to the study (8 males, 7 females). Subject characteristics are listed in Table 3.2. All subjects were accustomed to cycling between 3 - 6 hours in a single ride. Mean years of cycling training were 6.5 yr ± 1.2 yr. Written informed consent was obtained from each subject, and the study was approved by The University of Texas at Austin Institutional Review Board. Each subject served as his/her own control and performed the same protocol as shown in Figure 3.1 for each treatment.

Procedures

Preliminary testing. Prior to beginning the 2 experimental trials, subjects reported to the laboratory for determination of their VO_2max. The VO_2max tests and all experimental trials were performed on the same ergometer (Velotron Dynafit Pro, Racermate, Seattle, WA). The protocol for establishing VO_2max consisted of a 4 min warm up, then 2 min stages beginning at 200 watts (W) for males or 130 W for females. The workload was increased by 50 W (males) or 35 W (females) every 2 min until 350 W and 200 W, respectively. After this point, the workload increased 25 W (males) or 10 W (females) every minute until the subject could not continue to pedal despite constant verbal

encouragement. The criteria used to establish VO_2max was a plateau in VO_2 with increasing exercise intensity and RER > 1.10. During the test, subjects breathed through a Hans Rudolph valve, with expired gases directed to a mixing chamber for analysis of oxygen (O_2) and carbon dioxide (CO_2) (ParvoMedics TrueOne2400, ParvoMedics, Sandy, UT). Outputs from this system were directed to a laboratory computer for calculation of ventilation, O_2 consumption (VO_2), CO_2 production (VCO_2), and respiratory exchange ratio (RER) every 15 s.

Maximum power output in Watts was calculated from the VO_2max test data using the formula, adapted from Astrand and Rodahl (1):

$$W_{max} = (VO_2\text{max mL} - 300\text{ mL } O_2) / 12.5\text{ W/mL } O_2$$

The workloads were then set as percentages of the $Watts_{max}$ as follows:

$$W = [(VO_2\text{max mL} \times \%VO_2\text{max desired}) - 300\text{ mL } O_2] / 12.5\text{ W/mL } O_2$$

Each experimental trial was separated by a minimum of 7 days, not to exceed 14 days.

Ventilatory threshold (*post priori*). *Post priori*, we sought to determine if the intensity at which subjects cycled to exhaustion relative to their individual ventilatory thresholds contributed to the increases in TTE. Therefore, using the minute ventilation (V_E), VCO_2, and VO_2 data from the VO_2max test, ventilatory threshold (VT) was calculated using a computer-generated plot (ParvoMedics TrueOne2400 software). VT was defined as the point at which the V_E (minute ventilation) increased in a nonlinear fashion compared to increases in VO_2 and was substantiated by an increase in the V_E/VCO_2 to V_E/VO_2 ratio. Determination of VT was performed blinded. VO_2 was then calculated for the first 5 minutes of the performance part of the cycling protocol for each experimental trial and used to determine the percent of VT at which each subject was cycling. We then grouped the subjects as cycling at/below VT, or above VT *post priori*.

Cycling protocol. Three to five days after the VO_2max test, the subjects again reported to the laboratory to perform a familiarization ride, which also allowed verification and subsequent adjustment of the calculated workloads for the experimental trials. The familiarization ride followed exactly the same protocol as the experimental rides, except that no blood samples were collected, and only water was provided every 20 min. The protocol is shown in Figure 3.1. The first 30 min of the protocol were at low intensity (45% VO_2max). For the next 1.5 h, the intensity alternated every 8 min between 45% and 70% VO_2max. From hour 2 to 3, the intensity continued to alternate between 45% and 70% VO_2max, but did so every 3 min. After the 3 h time point, the intensity increased to between 74% and 85% of VO_2max (exhaustion protocol), and this marked the start of the TTE determination. Subjects were encouraged to ride as long as possible while maintaining a pedaling cadence of 80 to 90 revolutions per minute (rpm). When they could no longer maintain a pedaling cadence of 60 rpm despite constant verbal encouragement, they were asked to stop, and TTE was recorded as min:sec beyond the 3 h point. Constant verbal encouragement was given to the subjects during each trial, and the same investigators were present during all trials for each subject so that encouragement was consistent across all trials. In addition, subjects were not aware of how long they rode each time, as all timing devices were removed from their line of sight or covered.

For all trials, the subjects arrived at the laboratory after an overnight, 12 h fast, during which they were allowed to consume only water. Upon arrival, body weight was obtained and a heart rate monitor (Polar Beat, Polar Electro, Oy, Finland) was secured in place around the subject's chest. For the 2 experimental trials, a catheter fitted with a three-way stopcock and extended with a catheter extension was inserted into an antecubital vein and taped in place. A resting blood sample was taken as described below, and then the subject was given the first dose of supplement to drink. After consuming the 275 ml beverage, the subject mounted the ergometer and the cycling protocol began. Supplements (275 ml) were provided every 20 min for the duration of the ride. If the subjects were able to ride

longer than 40 min during the exhaustion portion of the protocol (i.e.; 40 min beyond the 3 h ride), and felt too full to continue to drink the entire amount of supplement provided each time, then they were asked to consume as much as they felt comfortable ingesting. During the exercise trials, the laboratory temperature was maintained at ~21° C and a fan was directed towards the subject to reduce thermal stress.

Diet and exercise. The subjects were instructed to maintain a training and dietary log for the 2 and 3 days, respectively, before the familiarization trial and to keep training and diet consistent with that record for the 2 and 3 days prior to the remaining experimental trials. The subjects provided a copy of their training and dietary logs on the day of the trials. An investigator reviewed and verified the entries in the logs with the subjects at each session in order to verify that compliance with the previous logs was attained. The data from the logs were entered into Nutribase Clinical Nutrition Manager 7.17 (CyberSoft, Inc., Phoenix, AZ) for nutritional analysis and compliance verification. All subjects complied with the diet and exercise requirements. Diets were not standardized across all subjects, as each subject served as his or her own control.

Blood sampling. Prior to receiving the first beverage dose and mounting the ergometer, a 5 ml sample of blood was collected and the catheter was flushed with saline. Five milliliters were drawn at 3 additional time points: at 118 min of exercise, at 177 min of exercise, and immediately after exercise ceased due to exhaustion. Saline flushes occurred every 10-15 min during the entire protocol to keep the catheter patent.

Ventilation, VO_2, RER, heart rate and RPE. Ventilation, VO_2, CO_2 production, and respiratory exchange ratio (RER) were recorded using the same ParvoMedics TrueOne 2400 system that was used during the VO_2max test and familiarization trial. The system was calibrated immediately before each trial using medical-grade gases of known concentrations and a 3.0 L calibration syringe. Collections were made at 4 time points: 10-15 min (low intensity), 46-51 min (high intensity), 130 – 136 min (low and high

intensity, 3 min each) and for the first 5 min of the exhaustion portion. With the exception of the 130-136 min collection, respiratory gases were collected for 5 min using 15 sec sampling, and only the last 1.5 min of each collection were used to determine steady state VO_2 and RER. For the 130-136 min collection (3 min of low intensity and 3 min of high intensity), the last minute of each interval was used. Heart rate (HR) was recorded at the beginning of exercise and at every 10-15 min of exercise. Subjective ratings of perceived exertion (RPE) on a Borg scale (ranging from 6 to 20) were obtained during exercise at the same time points as HR.

Substrate utilization. Determination of substrate (carbohydrate and fat) oxidation rates were made from VO_2, VCO_2, and RER values using the 5 collection times as described above during the experimental rides according to the method of Frayn (11).

Biochemical analyses of plasma metabolites. Each 5 ml blood sample taken during the protocol was anticoagulated with 0.3 ml of EDTA (24mg/ml, pH 7.4), and 0.5 ml of the anticoagulated blood was transferred to another tube containing 1 ml 10% perchloric acid (PCA). All tubes were centrifuged at 4˚ C for 10 min at 3,000 rpm with a HS-4 rotor in a Sorvall RC6 centrifuge (Kendro Laboratory Products, Newtown, CT). After centrifugation, plasma and PCA extracts were separated into aliquots for each assay and immediately frozen and stored at -80˚ C for later analysis. Plasma insulin was measured using ImmuChem^{TM} Coated Tube ^{125}I RIA Kit (MP Biomedicals, LLC, Orangeburg, NY) by radioimmunoassay (12). All samples were run in duplicate, with a coefficient of variation (CV) of 6.0%. Plasma myoglobin concentrations were determined by solid phase ELISA (BioCheck, Inc., Foster City, CA), and were run induplicate with a CV of 5.4%. Blood lactate was determined from the PCA extract by enzymatic-spectophotometric analysis based on the oxidation of lactate to pyruvate by nicotinamide adenine dicnucleotide (NAD^+) according to the method of Hohorst (13). Samples were run in duplicate and had a CV of 1.5%. Plasma glucose was measured using a spectophotometric Trinder reaction (no. 315, Sigma Chemical, St. Louis, MO). The

Trinder reagent contained the enzyme peroxidase (HPOD), 4-aminoantipyrine (4-AAP) and p-hydroxybenzene sulfonate (p-HBS). Glucose was oxidized to D-gluconate by glucose oxidase with production of an equal amount of hydrogen peroxide. Coupled by hydrogen peroxide, 4-AAP and p-HBS were oxidized by HPOD and formed a quinoneimine dye, intensely colored in red. The absorbance of the reaction solution was measured using a spectrophotometer (Beckman DU 640; Beckman Coulter, Inc., Fullerton, CA) at a wavelength of 500 nm. The intensity of the color in the reaction solution was proportional to the concentration of glucose in the plasma sample. Samples for this assay were also run in duplicate, with a CV of 3.7%.

Statistical Analyses

The data were analyzed using a general linear model for repeated measures. Time to exhaustion was analyzed using a one-way ANOVA. All the other variables that included multiple measures per trial were analyzed using a two-way ANOVA (treatment x time). *Post hoc* analysis was performed when significance was found using Fisher's Least Square Difference (LSD). The level of significance for all analyses was set at $P < 0.05$. All data are expressed as mean ± SEM. SPSS version 16.0 statistical software (SPSS Inc., Chicago, IL) was used for all statistical analyses.

RESULTS

Time to exhaustion. Time to exhaustion (TTE) for the combined group, as well as by VT grouping, is shown in Figure 3.2. For the combined group (n=15), TTE was not significantly different between treatments (MCP, 31.06 ± 5.76 vs CHO, 26.03 ± 4.27, p=0.064). However, for subjects cycling at or below VT (n=8), TTE in MCP was significantly greater than CHO (45.64 ± 7.38 vs 35.47 ± 5.94 min, p=0.006). There were no significant differences in TTE for the above VT group (n=7); CHO, 15.25 ± 2.83 vs MCP, 14.39 ± 2.50 min, p=0.8).

Blood and plasma analyses. Blood samples were analyzed for plasma insulin, plasma glucose, blood lactate and plasma myoglobin. No significant overall treatment differences in plasma insulin levels were found among treatments (Fig. 3.3). However, a significant treatment by time difference was observed at 177 min, with plasma insulin higher in CHO than in MCP in the combined group (p=.023), as well as in the at or below VT group (p=.032). No significant differences were found when exercising above VT.

Plasma glucose was significantly lower in MCP than CHO in the combined group (Fig. 3.4). Significant treatment by time effects were also observed at minutes 118 and 177 (p=.003 and .005, respectively). No treatment by time differences were found when grouped by VT.

Blood lactate levels rose significantly during the time to exhaustion portion of the protocol (Fig. 3.5), but there were no differences between the treatments whether grouped or not grouped by VT.

Plasma myoglobin levels rose during exercise in both treatments. Although myoglobin appeared to be lower at End in MCP, no significant differences were found between treatments (p=.189, Fig. 3.6). A similar non-significant trend was found in the group exercising above VT.

RPE and heart rate. Although RPE and HR values were recorded at the beginning of exercise and at every 10-15 min of exercise, 3 time points during low intensity intervals (90, 130 and 161 min), 2 time points during high intensity intervals (115 and 159 min), and 1 time point during the TTE protocol (184 min) were chosen for analysis. Values are shown in Table 3.3. There were no significant differences for RPE or HR between treatments regardless of VT grouping.

Substrate utilization. There were no significant treatment differences in either carbohydrate oxidation rates or fat oxidation rates ($g \cdot min^{-1}$) between MCP and CHO whether grouped or not grouped by VT (Table 3.4).

DISCUSSION

The primary objective of the present study was to compare the effects of a beverage containing a lower total amount, yet multiple types of carbohydrates, and a moderate amount of protein (3% CHO, 1.2% PRO) with a traditional 6% carbohydrate beverage on time to exhaustion (TTE) during prolonged endurance exercise. The most important finding of this investigation was that TTE was significantly greater in the treatment that contained fewer calories, lower carbohydrates, and a moderate amount of protein (MCP), compared to the higher carbohydrate-containing beverage (CHO) when exercising at VT or ~2% below VT (MCP, 45.64 ± 7.38 vs CHO, 35.47 ± 5.94 min, p=0.006). This represents a 28.7% improvement in TTE in the MCP treatment. In the combined group of 15 subjects, TTE in MCP was greater than CHO by 19.3% (31.06 ± 5.76 vs 26.03 ± 4.27, p=0.064), although it did not reach statistical significance. When exercising above VT by 5-7%, there was no difference in TTE between treatments (15.25 ± 2.83 vs MCP, 14.39 ± 2.50 min, p=0.8).

Our finding of differences when grouped according to VT (e.g., cycling at or below VT or cycling above VT during the exhaustion protocol) is novel. Many investigations report TTE when cycling at a work rate that elicits a certain percentage of VO_2max, often 70% - 85% VO_2max (26-28,30), without identifying how this percentage relates to the subjects' ventilatory thresholds (VT) or lactate thresholds (LT). Recently, other investigators have pointed out that the relative intensity at which individuals are exercising could be a factor in determining the efficacy of the CHO and CHO+PRO supplements (4). Indeed, the ability to exercise for long periods near an individual's LT becomes a critical component of performance in long events such as marathons, longer cycling races and long-distance triathlons. In fact, it has been shown that runners self-select a running pace just above the

point of blood lactate appearance and are able to maintain that intensity for the duration of a marathon (10). In the present study, we utilized ventilatory threshold (VT) *post-priori* as a surrogate for LT, given that VT and LT have been shown to occur near the same VO_2 (8,16). The significant differences found in TTE when cycling at or below VT suggest that MCP is more effective in extending endurance and delaying fatigue than CHO around the exercise intensity at which prolonged endurance performance is often crucial. While we did not observe differences in TTE when exercising above VT, it is possible that other factors that contribute to exhaustion when exercising at higher intensities, such as a significant drop in muscle pH or depletion of high energy phosphates, simply may not be affected by the supplement.

Many investigations have demonstrated improved TTE with the addition of protein to a carbohydrate supplement ingested during exercise (15,27,28), although the supplements are usually isocaloric or isocarbohydrate compared to a 6-8% CHO supplement (15,27). However, Martinez-Lagunas et al. (24) recently compared the effects of a 4.5% CHO plus 1.15% PRO, and a 3% CHO plus 0.75% PRO beverage to a traditional 6% CHO beverage. The investigators found that while TTE was not significantly different between treatments, both of the lower CHO plus PRO treatments still maintained TTE performance. Although their investigation used a similar exercise protocol and low CHO+PRO/low calorie treatment as were utilized in the present study, there are methodological differences that may have contributed to their non-significant differences in TTE. First, the Martinez-Lagunas et al. (24) investigation did not take into consideration the relative intensity at which the subjects were exercising. Second, Martinez-Lagunas and colleagues (24) used a single carbohydrate (dextrose) rather than a mixture of different carbohydrates as was employed in the present study.

The use of three different carbohydrates in the MCP beverage used in the present investigation may have enabled the optimization of the various intestinal carbohydrate transporters such that the rate of absorption was increased beyond that of the single-CHO

treatment, leading to increased exogenous CHO oxidation and decreased endogenous CHO oxidation. Several previous investigations have demonstrated that, compared to a single CHO supplement, supplements containing mixtures of carbohydrates can increase exogenous CHO oxidation (17-20,32), decrease endogenous CHO oxidation (17,19), and improve cycling exercise performance (7). Recently, Currell and Jeukendrup (7) compared the effects of a CHO mixture to a single CHO supplement on cycling performance. Subjects cycled for 120 min at 55% W_{max} while ingesting a glucose-only supplement, an isocaloric glucose plus fructose supplement, or water, followed by a time trial in which the subjects had to complete a set amount of work as quickly as possible. Ingestion of the glucose plus fructose supplement resulted in an 8% faster time trial time compared to glucose only, and 19% faster than water (7). Total CHO oxidation was not different between the CHO treatments, and the investigators concluded that ingestion of the CHO mixture likely led to a sparing of endogenous CHO stores, since glucose plus fructose has been shown to have a greater exogenous CHO oxidation than glucose only (18,19). Taken together, these findings suggest that the sparing of endogenous CHO may be a key mechanism that resulted in the TTE improvements reported here.

Others, however, have failed to show that CHO+PRO supplementation during exercise spares endogenous fuel supplies by decreasing muscle glycogen use or depletion of Krebs cycle intermediates (4). Cermak and colleagues (4) found no difference in muscle glycogen use or in levels of Krebs cycle intermediates when trained men ingested 6% CHO or 6% + 2% PRO during 90 min of cycling at ~69% VO_2peak. It should be noted, however, that the investigators used a single carbohydrate in both treatments rather than a mixture of carbohydrates as was used in the present study. It is possible that using a mixture of carbohydrates in a CHO+PRO supplement could demonstrate a positive effect in the levels of muscle glycogen use and Krebs cycle intermediates compared to a supplement containing a single carbohydrate with added protein, although we did not measure these parameters in the present study.

Other investigators have proposed that reducing muscle damage that occurs during intense endurance exercise is a possible mechanism for improved performance with CHO+PRO supplementation. Saunders and colleagues (27,28) have demonstrated significant improvements in TTE concomitant with significant reductions in markers of muscle damage when comparing CHO+PRO supplements to CHO only supplements (27,28). Others have used a resistance exercise model to demonstrate a significant reduction in myoglobin levels 6 h after a strenuous resistance exercise bout when subjects ingested a CHO+PRO beverage compared to a placebo supplement (2). However, other recent investigations have demonstrated significant reductions in muscle damage without differences in TTE (26,30).

In the present investigation, however, we did not demonstrate a statistically significant reduction in myoglobin. Myoglobin was selected as our muscle damage marker of choice because it is a small molecule that leaks from the skeletal muscle cell early on during exercise when damage occurs, peaking in about 1 h post-exercise (9,29). In the combined group (Fig. 3.6), myoglobin levels continued to rise over the course of the trial with the CHO treatment, whereas with MCP, myoglobin increased to a lesser but non-significant extent ($p=.189$). It is possible that had we evaluated myoglobin again within hours post-exercise rather than immediately at the end of exercise (e.g., at exhaustion), a greater difference in myoglobin levels may have been detected. A trend was observed for an increasing difference in myoglobin levels between treatments, yet without a measurement an hour post-exercise, we do not know if that trend would have continued, resulting in a significant difference. Therefore, although we cannot associate the improvement in TTE observed in the present study with muscle damage reduction, the possibility remains that reduced muscle damage could potentially help to maintain or improve performance.

In summary, proper nutritional supplementation during endurance exercise is essential for delaying fatigue and maintaining optimal performance. Most of the commercially available products contain high total calories and large amounts of carbohydrate. Many

athletes and recreational exercisers want to maintain or improve their body composition, and are therefore concerned about limiting caloric intake. The present investigation demonstrates that consuming a beverage containing a mixture of different carbohydrates, a moderate amount of protein and fewer calories than a traditional, higher single-carbohydrate supplement during endurance exercise can extend exercise time to exhaustion, especially when exercising at or below the ventilatory threshold. This is an important finding that is highly relevant to endurance athletes and exercisers; especially those who wish to consume less total carbohydrate and fewer calories, and improve endurance capacity. The present study demonstrated that a supplement containing a mixture of carbohydrates plus a moderate amount of protein could significantly improve aerobic endurance when cycling at or below the ventilatory threshold, despite containing 50% less total carbohydrate and 30% fewer calories relative to a higher carbohydrate beverage.

REFERENCES

1. Astrand PO and Rodahl K. Textbook of Work Physiology**,** 2nd edn, McGraw-Hill Book. Co, New York, 1977.
2. Baty JJ, Hwang H, Ding Z, Bernard JR, Wang B, Kwon B, and Ivy JL. The effect of a carbohydrate and protein supplement on resistance exercise performance, hormonal response, and muscle damage. J Strength Cond Res 21: 321–329, 2007.
3. Calders P, Matthys D, Derave W, and Pannier JL. Effect of branched-chain amino acids (BCAA), glucose, and glucose plus BCAA on endurance performance in rats. Med Sci Sports Exerc 31: 583-587, 1999.
4. Cermak NM, Solheim AS, Gardner MS, Tarnopolsky MA, and Gibala MJ. Muscle metabolism during exercise with carbohydrate or protein-carbohydrate ingestion. Med Sci Sports Exerc 41: 2158-2164, 2009.
5. Coggan AR, and Coyle EF. Effect of carbohydrate feedings during high-intensity exercise. J Appl Physiol 65: 1703-1709, 1988.
6. Coggan AR, and Coyle EF. Reversal of fatigue during prolonged exercise by carbohydrate infusion or ingestion. J Appl Physiol 63: 2388-2395, 1987.
7. Currell K, and Jeukendrup AE. Superior endurance performance with ingestion of multiple transportable carbohydrates. Med Sci Sports Exerc 40: 275-281, 2008.
8. Davis JA, Vodak P, Wilmore JH, Vodak J, and Kurtz P. Anaerobic threshold and maximal aerobic power for three modes of exercise. J Appl Physiol 41: 544-550, 1976.
9. Driessen-Kletter MF, Amelink GJ, Bar PR, and van Gijn J. Myoglobin is a sensitive marker of increased muscle membrane vulnerability. J Neurol 237: 234-238, 1990.
10. Farrell PA, Wilmore JH, Coyle EF, Billing JE, and Costill DL. Plasma lactate accumulation and distance running performance. Med Sci Sports 11: 338-344, 1979.
11. Frayn KN. Calculation of substrate oxidation rates in vivo from gaseous exchange. J Appl Physiol 55: 628-634, 1983.
12. Goetz FC, and Greenberg BZ. A simple immunoassay for small amounts of insulin. J Clin Lab Med 58: 819-822, 1961.
13. Hohorst HJ. Determination of L-lactate with LDH and DPN. In: Methods of Enzymatic Analysis, edited by Bergmeyer HU. New York: Academic, 1963, p. 266-270.
14. Ivy JL, Miller W, Dover V, Goodyear LG, Sherman WM, Farrell S, and Williams H. Endurance improved by ingestion of a glucose polymer supplement. Med Sci Sports Exerc 15: 466-471, 1983.
15. Ivy JL, Res PT, Sprague RC, and Widzer MO. Effect of a carbohydrate-protein supplement on endurance performance during exercise of varying intensity. Int J Sport Nutr Exerc Metab 13: 382-395, 2003.
16. Ivy JL, Withers RT, Van Handel PJ, Elger DH, and Costill DL. Muscle respiratory capacity and fiber type as determinants of the lactate threshold. J Appl Physiol 48: 523-527, 1980.

17. Jentjens RL, Achten J, and Jeukendrup AE. High oxidation rates from combined carbohydrates ingested during exercise. Med Sci Sports Exerc 36: 1551-1558, 2004a.
18. Jentjens RL, and Jeukendrup AE. High rates of exogenous carbohydrate oxidation from a mixture of glucose and fructose ingested during prolonged cycling exercise. Br J Nutr 93: 485-492, 2005.
19. Jentjens RL, Moseley L, Waring RH, Harding LK, and Jeukendrup AE. Oxidation of combined ingestion of glucose and fructose during exercise. J Appl Physiol 96: 1277-1284, 2004b.
20. Jentjens RLPG, Venables MC, and Jeukendrup AE. Oxidation of exogenous glucose, sucrose, and maltose during prolonged cycling exercise. J Appl Physiol 96: 1285-1291, 2004c.
21. Jeukendrup AE. Carbohydrate intake during exercise and performance. Nutrition 20: 669-677, 2004.
22. Jeukendrup AE, and Jentjens R. Oxidation of carbohydrate feedings during prolonged exercise: current thoughts, guidelines and directions for future research. Sports Med 29: 407-424, 2000.
23. Jeukendrup AE, Wagenmakers AJM, Stegen JHCH, Gijsen AP, Brouns F, and Saris WHM. Carbohydrate ingestion can completely suppress endogenous glucose production during exercise. Am J Physiol Endocrinol Metab 276: E672-683, 1999.
24. Martinez-Lagunas V, Ding Z, Bernard JR, Wang B, and Ivy JL. Added Protein Maintains Efficacy of a Low-Carbohydrate Sports Drink. J Strength Cond Res 2009.
25. Niles ES, Lachowetz T, Garfi J, Sullivan W, Smith JC, Leyh BP, and Headley SA. Carbohydrate-Protein Drink Improves Time to Exhaustion after Recovery from Endurance Exercise. JEPonline 4: 45-52, 2001.
26. Romano-Ely B, Todd M, Saunders M, and St. Laurent T. Effect of an Isocaloric Carbohydrate-Protein-Antioxidant Drink on Cycling Performance. Med Sci Sports Exc 38: 1608-1616, 2006.
27. Saunders M, Kane M, and Todd K. Effects of a Carbohydrate-Protein Beverage on Cycling Endurance and Muscle Damage. Med Sci Sports Exc 36: 1233-1238, 2004.
28. Saunders MJ, Luden ND, and Herrick JE. Consumption of an oral carbohydrate-protein gel improves cycling endurance and prevents postexercise muscle damage. J Strength Cond Res 21: 678-684, 2007.
29. Sorichter S, Mair J, Koller A, Pelsers MM, Puschendorf B, and Glatz JF. Early assessment of exercise induced skeletal muscle injury using plasma fatty acid binding protein. Br J Sports Med 32: 121-124, 1998.
30. Valentine RJ, Saunders MJ, Todd MK, and St Laurent TG. Influence of carbohydrate-protein beverage on cycling endurance and indices of muscle disruption. Int J Sport Nutr Exerc Metab 18: 363-378, 2008.

31. Wagenmakers AJ, Brouns F, Saris WH, and Halliday D. Oxidation rates of orally ingested carbohydrates during prolonged exercise in men. J Appl Physiol 75: 2774-2780, 1993.
32. Wallis GA, Rowlands DS, Shaw C, Jentjens RL, and Jeukendrup AE. Oxidation of combined ingestion of maltodextrins and fructose during exercise. Med Sci Sports Exerc 37: 426-432, 2005.
33. Williams MB, Raven PB, Fogt DL, and Ivy JL. Effects of Recovery Beverages on Glycogen Restoration and Endurance Exercise Performance. Journal of Strength and Conditioning Research 17: 12-19, 2003.
34. Yaspelkis BB, 3rd, and Ivy JL. Effect of carbohydrate supplements and water on exercise metabolism in the heat. J Appl Physiol 71: 680-687, 1991.
35. Yaspelkis BB, 3rd, Patterson JG, Anderla PA, Ding Z, and Ivy JL. Carbohydrate supplementation spares muscle glycogen during variable-intensity exercise. J Appl Physiol 75: 1477-1485, 1993.

	CHO	MCP
Kcals	24.0	16.9
% total CHO	6.0	3.0
% dextrose	6	1
% fructose	0	1
% maltodextrin	0	1
% PRO	0	1.2
Ratio of CHO:PRO	--	2.5:1
CHO g	6	3
PRO g	0	1.2

Per 100 ml. Both treatments contained the same amounts of electrolytes NA^{+}, K^{+}, and MG^{++}. 275 ml was provided immediately before exercise and every 20 min thereafter.

Table 3.1. Energy and macronutrient content of the treatments

	Age (yr)	Mass (kg)	Height (cm)	VO_2max ($L \cdot min^{-1}$)	VT ($L \cdot min^{-1}$)
Mean, n = 15	28.7 ± 1.2	65.9 ± 2.4	172.0 ± 2.2	3.70 ±.20	2.80 ± .20
Males, n = 8	28.9 ± 1.9	69.2 ± 2.5	175.8 ± 2.0	4.50 ± .10	3.40 ± .10
Females, n = 7	28.6 ± 1.5	62.1 ± 4.1	167.7 ± 3.6	2.90 ± .20	2.00 ± .20
At or below VT, n=8	29.5 ± 1.2	64.5 ± 2.5	172.6 ± 3.6	3.50 ± .30	2.70 ± .20
Above VT, n=7	27.9 ± 2.2	67.4 ± 4.5	171.3 ± 2.6	4.00 ± .40	3.00 ± .30

Data are presented as mean ± SE.

Table 3.2. Subject characteristics

			MCP			CHO		
	Intensity	Min	Combined Group (n=15)	=/< VT (n=8)	> VT (n=7)	Combined Group (n=15)	=/< VT (n=8)	> VT (n=7)
RPE	Low	90	11 ± .31	12 ± .46	11 ± .40	11 ± .33	11 ± .36	11 ± .57
	Low	130	12 ± .28	12 ±.50	11 ± .20	12 ±.30	12 ± .53	11 ± .18
	Low	161	12 ± .35	12 ± .55	12 ± .43	12 ± .33	12 ± .44	11 ± .41
	High	115	14 ± .22	14 ± .27	13 ± .36	14 ± .31	14 ± .38	14 ± .53
	High	159	14 ± .34	14 ± .31	14 ± .65	14 ± .37	14 ± .45	14 ± .61
	High/TTE	184	16 ± .32	16 ±.41	16 ± .53	16 ± .30	16 ±.42	16 ± .43
HR	Low	90	119.4 ± 3.3	121.9 ± 5.1	116.6 ± 4.0	120.9 ± 2.9	123.9 ± 3.8	117.4 ± 4.5
	Low	130	124.9 ± 3.3	126.8 ± 4.9	122.9 ± 4.6	127.6 ± 3.8	130.6 ± 5.7	124.1 ± 5.0
	Low	161	130.0 ± 3.5	129.4 ± 5.2	130.7 ± 5.2	132.1 ± 3.5	134.0 ± 5.0	129.9 ± 5.1
	High	115	143.7 ± 4.2	143.5 ± 7.0	144.0 ± 4.9	148.7 ± 3.9	148.8 ± 5.7	148.6 ± 5.7
	High	159	145.1 ± 4.3	143.5 ± 7.2	147.0 ± 4.5	149.3 ± 3.5	149.4 ± 4.9	149.1 ± 5.3
	High/TTE	184	162.7 ± 3.8	160.5 ± 5.8	165.3 ± 4.9	162.9 ± 3.7	160.5 ± 5.2	165.6 ± 5.5

Values are mean ± SE.

Table 3.3. RPE and heart rate during cycling protocol

		MCP			CHO		
Substrate	Intensity	Combined Group (n=15)	=/< VT (n=8)	> VT (n=7)	Combined Group (n=15)	=/< VT (n=8)	> VT (n=7)
CHO ($g \cdot min^{-1}$)	Low	1.50 ± .11	1.44 ± .12	1.59 ± .20	1.54 ± .11	1.47 ± .13	1.64 ± .19
	High	2.67 ± .19	2.53 ± .24	2.84 ± .30	2.70 ±.19	2.50 ± .23	2.94 ± .30
	High/TTE	3.56 ± .25	3.18 ± .29	3.99 ± .38	3.65 ± .27	3.25 ± .31	4.11 ± .40
Fat ($g \cdot min^{-1}$)	Low	0.24 ± .03	0.22 ± .03	0.28 ± .06	0.21 ± .02	0.20 ± .02	0.23 ± .04
	High	0.19 ± .03	0.18 ± .05	0.23 ± .06	0.17 ± .03	0.17 ± .05	0.17 ± .04
	High/TTE	0.06 ± .02	0.08 ±.04	0.05 ± .03	0.02 ± .02	0.05 ± .03	0.00 ± .00

Values are mean ± SE.

Table 3.4. Substrate utilization during cycling protocol

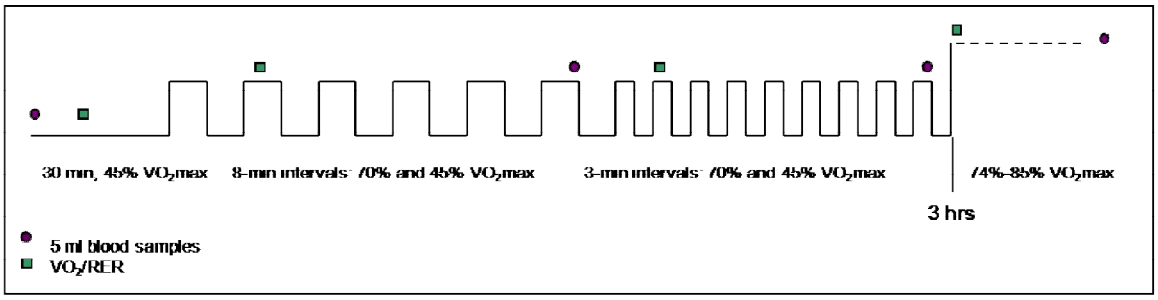

Figure 3.1. Experimental protocol

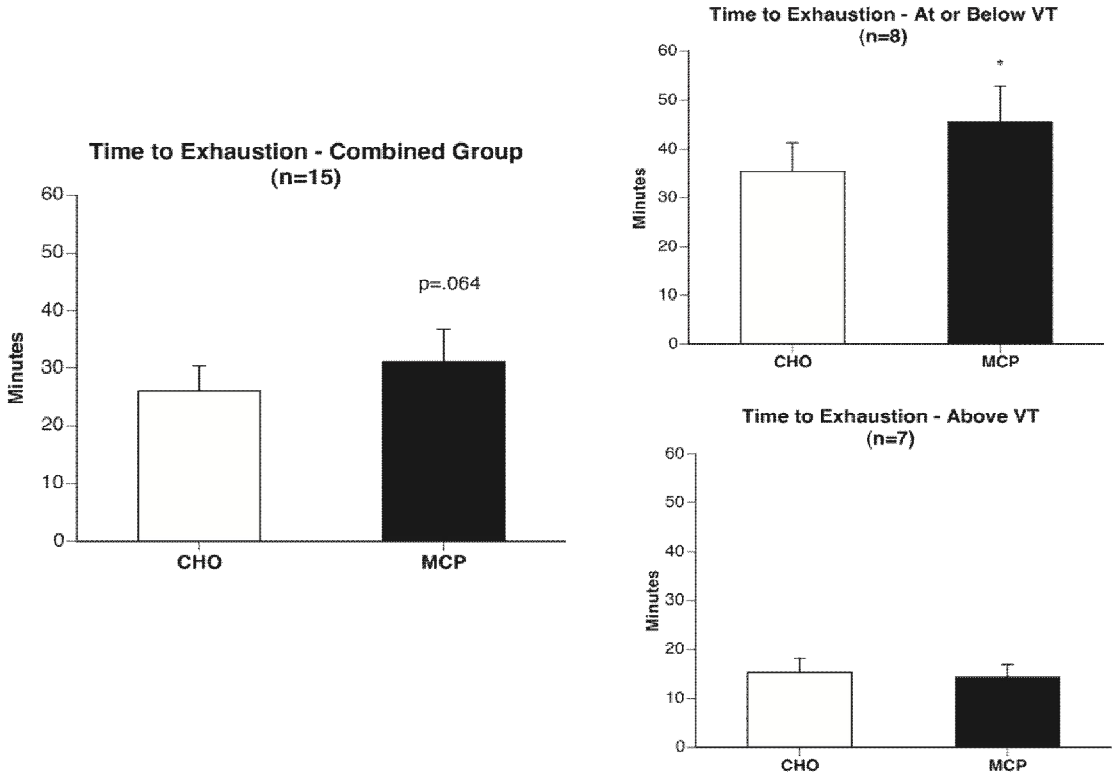

Figure 3.2. Time to exhaustion. Time to exhaustion (TTE) for (a) the combined group (n=15), (b) at or below VT (n=8), and (c) above VT (n=7). *Significantly different than CHO (p=.006). Values are mean ± SE.

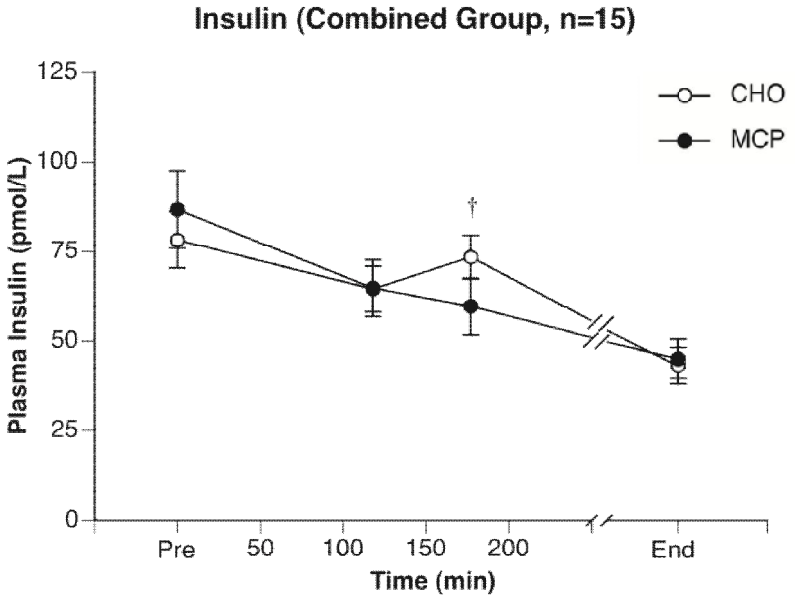

Figure 3.3. Plasma insulin. Insulin was measured from blood samples taken at rest, at the end of the 8-min intervals (118 min) at the end of the 3 min intervals (177 min) and at exhaustion. †Significantly higher than MCP at 177 min in the combined group (p=.023). Insulin was also higher in the CHO treatment at minute 177 in the group exercising at or below VT (p=.032) but no differences were observed above VT (data not shown). A significant time effect was found in both treatments (p=.000 for both). Values are mean ± SE.

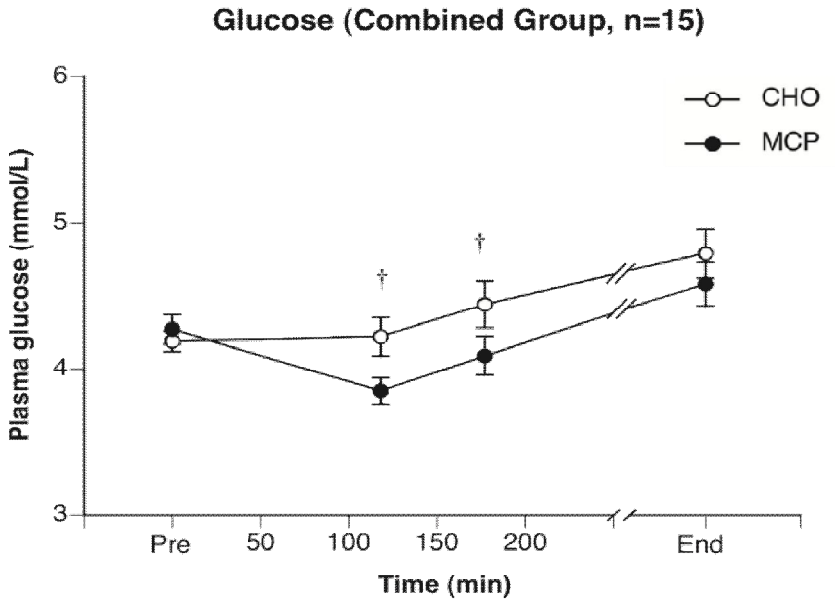

Figure 3.4. Plasma glucose. Glucose was measured from blood samples taken at rest, at the end of the 8-min intervals (118 min) at the end of the 3 min intervals (177 min) and at exhaustion. †Significantly higher than MCP at 118 and 177 min in the combined group (p=.003 and .005, respectively). A significant time effect was present in both treatments (p=.000 for both) in the combined group, but only existed for the CHO treatment when grouped by VT (at or below VT, p=.008; above VT, p=.033). Values are mean ± SE.

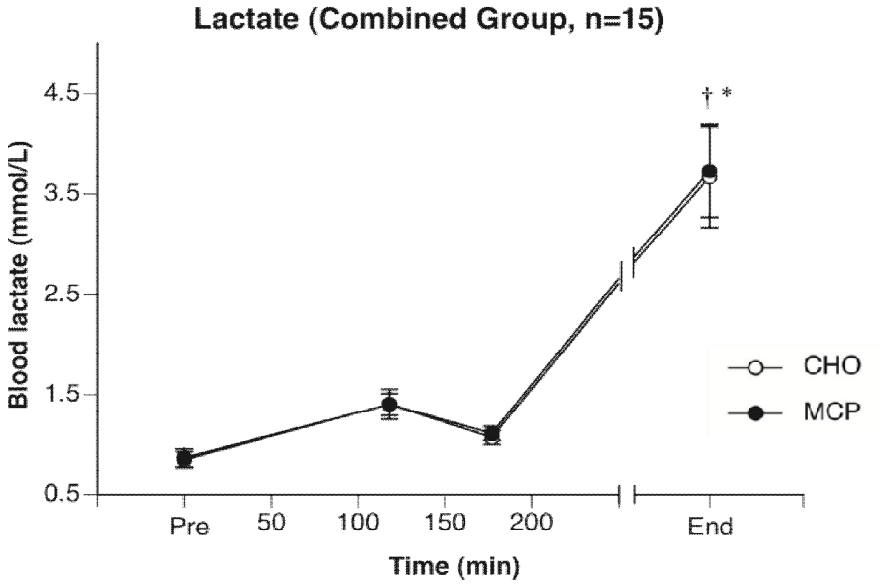

Figure 3.5. Blood lactate. Lactate was measured from blood samples taken at rest, at the end of the 8-min intervals (118 min) at the end of the 3 min intervals (177 min) and at exhaustion. A significant time effect was observed in both treatments, as blood lactate rose during the exhaustion protocol (†CHO, p=.000; *MCP, p=.000). At or below VT, as well as above VT, there was also a significant time effect (data not shown) (p=<.05 for both treatments). Values are mean ± SE.

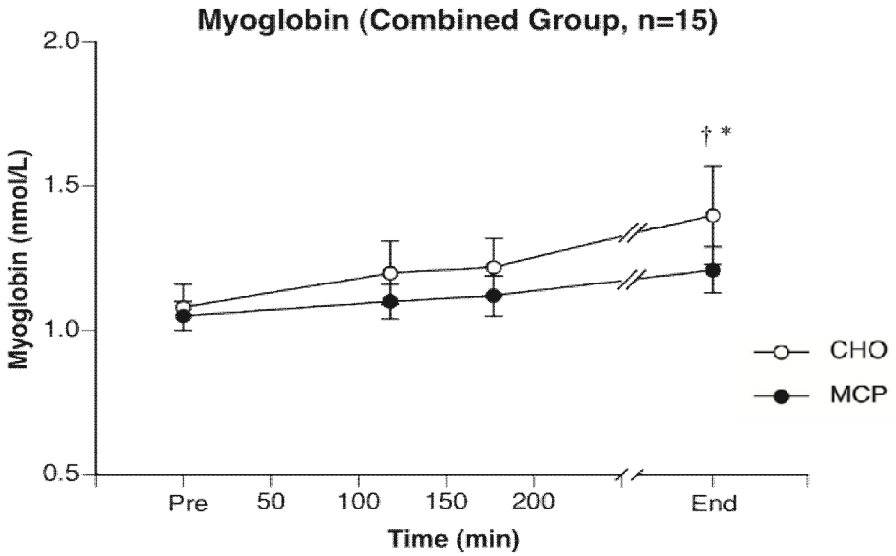

Figure 3.6. Plasma myoglobin. Myoglobin was measured from blood samples taken at rest, at the end of the 8-min intervals (118 min) at the end of the 3 min intervals (177 min) and at exhaustion. A significant time effect was observed in the combined group (†CHO, p=.001; *MCP, p=.005). Values are mean ± SE.

Chapter IV: Post-Exercise Carbohydrate-Protein Supplementation Improves Subsequent Exercise Performance and Intracellular Signaling for Protein Synthesis

ABSTRACT

Post-exercise carbohydrate-protein supplementation has been proposed to improve recovery and subsequent endurance performance compared to carbohydrate. This study compared the effects a carbohydrate-protein supplement in the form of chocolate milk (CM), isocaloric carbohydrate (CHO), and placebo (PLA) on recovery and subsequent exercise performance. Ten cyclists performed 3 trials, cycling 1.5 h at 70% VO_2max plus 10 min of intervals. They ingested supplements immediately post-exercise and 2 h into a 4-h recovery. Biopsies were performed at recovery minutes 0, 45 and 240 (R0, R45, REnd). Post-recovery, subjects performed a 40 km time trial (TT). TT time was faster in CM than CHO and PLA (79.43±2.11 vs 85.74±3.44 and 86.92±3.28 min, $P<0.05$). Muscle glycogen resynthesis was higher in CM and CHO than PLA (23.58 and 30.58 vs 7.05 μmol/g wet wt, $P<0.05$). mTOR phosphorylation was greater at R45 in CM than CHO or PLA (174.4 ± 36.3 vs. 131.3 ± 28.1 and 73.7 ± 7.8% standard, $P<0.05$), and at REnd in CM than PLA (94.5 ± 9.9 vs 69.1 ± 3.8%, $P<0.05$). rpS6 phosphorylation was greater in CM than PLA at R45 (41.0 ± 8.3 vs 15.3 ± 2.9%, $P<0.05$) and REnd (16.8 ± 2.8 vs 8.4 ± 1.9%, $P<0.05$). FOXO3A phosphorylation was greater at R45 in CM and CHO than PLA (84.7 ± 6.7 and 85.4 ± 4.7 vs 69.2 ± 5.5, $P<0.05$). These results indicate that chocolate milk is an effective post-exercise supplement that can improve subsequent exercise performance and provide a greater intracellular signaling stimulus for protein synthesis compared to carbohydrate and placebo.

INTRODUCTION

Recovering from strenuous endurance exercise is central to the ability to perform at one's best day after day in training sessions and competitive events. Endurance athletes must often perform multiple bouts of prolonged, strenuous activity with little time to recover in between. Studies of subsequent endurance exercise performance performed after recovering from a previous bout have reported significantly increased endurance time to exhaustion in the subsequent exercise bout with carbohydrate-protein supplementation (CHO+PRO) compared to carbohydrate (CHO) alone (3, 34, 45, 56). The improvements in performance have been associated with a greater recovery of muscle glycogen during the recovery period between the two bouts in CHO+PRO compared to a non-isocaloric CHO beverage (4, 56), as well as with reduced muscle damage (45). However, not all investigations have demonstrated a significant difference in subsequent time to exhaustion (36, 42-44), or time trial performance (4, 7, 8) between CHO+PRO and CHO treatments.

In addition to the importance of post-exercise supplementation in restoring muscle glycogen and attenuating muscle damage for improved recovery, nutrients play a key role in facilitating muscle protein synthesis. Strenuous exercise stimulates protein synthesis as well as protein breakdown, and the balance between degradation and synthesis is largely mediated by nutrient availability, especially that of amino acids (AA), and activation of the mTOR (mammalian target of rapamycin) signal transduction pathway. mTOR is a serine/threonine kinase that integrates signals from nutrients, skeletal muscle contraction and growth factors, functioning as a crucial regulator of protein synthesis (30, 52). Because the mTOR pathway is critical for protein synthesis and cell growth, it is important to optimize activation of this pathway in order to maximize training adaptations. While post-exercise CHO+PRO supplementation has been shown to alter the phosphorylation of signaling proteins related to protein synthesis (20, 32), the effects when compared to CHO only and placebo in an endurance exercise model has not been

well characterized, nor has muscle protein degradation-specific signaling been characterized in response to CHO+PRO supplementation post-endurance exercise.

While there are many CHO+PRO supplements commercially available, several recent investigations have used low-fat chocolate milk (CM) as a post-endurance exercise CHO+PRO supplement. Pritchett and colleagues compared CM and a commercially available CHO+PRO post-exercise supplement and found no difference between the two in markers of muscle damage and in time to exhaustion in a subsequent bout of exercise (39). This finding was in agreement with another investigation that compared CM to a CHO-replacement beverage and a fluid-replacement beverage, and found that time to exhaustion (TTE) after a 4 h recovery was significantly longer in CM than the other 2 treatments (49). Taken together, these findings suggest that CM may be a viable CHO+PRO supplement for use post-exercise to aid recovery, improve subsequent exercise performance, activate signaling pathways for initiation of protein translation, while also reducing protein degradation signaling beyond that afforded by CHO supplementation alone. However, to our knowledge, no single investigation has examined all of these aspects together, along with markers of muscle damage, inflammation, and muscle glycogen resynthesis.

Therefore, the purpose of this study was to investigate the effects of a dairy-based carbohydrate-protein recovery supplement (chocolate milk, CM) on post-exercise muscle glycogen synthesis, activation of key signaling proteins involved in protein synthesis (mTOR, rpS6, and eIF2Bε) and degradation (FOXO3A and ubiquitination), biochemical measures of muscle damage and inflammation, and subsequent aerobic endurance performance. We hypothesized that, compared to an isocaloric CHO supplement, the CM supplement would improve recovery by increasing muscle glycogen synthesis and attenuate indicators of muscle damage and inflammation after a strenuous exercise bout and thus, improved subsequent time trial performance. We also hypothesized that the CM supplement would more effectively modulate the phosphorylation of intracellular

signaling proteins that lead to increased protein synthesis and reduced protein degradation during recovery compared to a CHO supplement or placebo.

METHODS

Subjects. Ten healthy, trained cyclists and triathletes (5 males, 5 females) between the ages of 18 and 39 years were admitted to the study. Subject characteristics are listed in Table 4.1. Written informed consent was obtained from all subjects, and the study was approved by The University of Texas at Austin Institutional Review Board.

Research design. This study followed a randomized, double-blinded, placebo-controlled, crossover design. Subjects reported to the laboratory before the start of their experimental trials for maximal oxygen consumption (VO_2max) and maximal workload (Wmax) determination. This test was performed on a VeloTron DynaFit Pro cycle ergometer (RacerMate, Seattle, WA). The protocol for establishing VO_2max consisted of a 4 min warm up, then 2 min stages beginning at 200 watts (W) for males or 130 W for females. The work load was increased by 50 W (males) or 35 W (females) every 2 min until 350 W and 200 W, respectively. After this point, the workload increased 25 W (males) or 10 W (females) every minute until the subject could not continue to pedal despite constant verbal encouragement. The criteria used to establish VO_2max was a plateau in VO_2 with increasing exercise intensity and RER > 1.10. VO_2max was measured using a TrueOne2400 system (ParvoMedics, Sandy, UT). Subjects breathed through a Hans Rudolph valve, with expired gases directed to a mixing chamber for analysis of oxygen (O_2) and carbon dioxide (CO_2). Outputs were directed to a computer for calculation of ventilation, O_2 consumption (VO_2), CO_2 production (VCO_2), and respiratory exchange ratio (RER) every 15 s.

Maximum power output in Watts was calculated from the VO_2max test data using the formula, adapted from ó strand and Rodahl (1):

$$W_{max} = (VO_2max\ mL - 300\ mL\ O_2) / 12.5\ W/mL\ O_2$$

The workload for each intensity level (45%, 70% and 90% of VO_2max) was then set as percentages of the W_{max} as follows:

$$W = [(VO_2max\ mL \times \%VO_2max\ desired) - 300\ mL\ O_2] / 12.5\ W/mL\ O_2$$

Three to five days after the VO_2max test, the subjects again reported to the laboratory after an overnight 12-h fast for a familiarization session. Using the same VeloTron cycle ergometer on which the VO_2max test was performed, subjects performed a 40 min portion of the glycogen depletion ride, including the intervals at the end, and gas collections taken during this ride allowed verification and subsequent adjustment of the calculated workloads for the experimental trials. Subjects then rested in the laboratory for 1 h, and then performed the 40 km cycling time trial. No muscle or blood samples were collected during the familiarization session.

The protocol for the experimental trials is shown in Figure 4.1. For each trial, subjects reported to the laboratory having fasted overnight for 12 h. After being weighed and fitted with a heart rate monitor (Cardiosport, Waterlooville, Hampshire, UK), a catheter with a 3-way stopcock and catheter extension was inserted into a forearm vein and taped in place. A resting blood sample was drawn, and resting heart rate recorded. Then, the subject mounted the cycle ergometer and began the glycogen depletion ride.

Subjects cycled at 70% VO_2max for 1.5 h, followed by 1 min alternating intervals at 45% and 90% VO_2max for a total of 10 min. This bout was designed to deplete muscle glycogen stores. Subjects received 250 mL of water at 15 min intervals during the ride, and floor fans were used to circulate air over the individual to minimize thermal stress. Subjects then recovered in the laboratory for 4 h and received one of 3 experimental treatments immediately and at 2-h post-exercise. Muscle biopsies were performed

immediately following the first exercise bout, at 45 min into recovery, and 4 h after the start of recovery to assess muscle glycogen resynthesis. Blood collections were taken at the same time points as the biopsies, as well as at 2 h into the recovery period. Subjects were allowed to work, read, or study at a desk set up for their use in the laboratory, and were under constant observation by investigators during this time. They were provided water *ad libitum* during this time. At four time points during recovery, RER measurements were made while the subject rested quietly in a cushioned chair for 10 min. The catheters were flushed every 10 - 15 min throughout the recovery period.

Following the recovery period, subjects completed a 40-km time trial (TT) using the same VeloTron on which they performed the earlier exercise bout. The simulated TT course was designed with VeloTron 3D software (RacerMate, Seattle, WA). The subjects were instructed to cycle this fixed distance as fast as possible, and the measure of performance was the time to complete the TT. The course included rolling hills and a 3-km uphill finish. Subjects were fan cooled and were provided 250 ml of water at 15 min intervals during the TT. Verbal encouragement was given consistently to all subjects, and the same investigators provided encouragement to the same subjects for each TT. Each experimental trial was separated by a minimum of 7 days, but did not exceed 14 days.

Experimental beverages. The 3 treatment beverages were chocolate milk (CM; Kirkland Organic Low-Fat Chocolate Milk, Costco Inc.), carbohydrate (CHO; dextrose and canola oil) or placebo (PLA; water flavored with Splenda and non-caloric Kool Aid flavoring). Energy and macronutrient composition of the beverages is shown in Table 4.2. The CM and CHO were isocaloric. The supplements were provided immediately post-exercise after the first biopsy was performed, and again 2 h later. Subjects were instructed to drink the amount provided within 5 min. The amounts of supplement provided were stratified according to body weight ranges. Subjects weighing <63.6 kg (140 lbs) received 500 mL per supplement (395.25 kcals each), totaling 1000 mL and 790.5 kcals during the recovery period. Subjects weighing between 63.6 kg (140 lbs) and 77.2 kg (170 lbs)

received 600 mL per supplement (474.3 kcals), totaling 1200 mL and 948.6 kcals during recovery. Subjects weighing >77.2 kg (170 lbs) received 700 mL per supplement (553.35 kcals), totaling 1400 mL and 1106.7 kcals during recovery. For the carbohydrate treatment, the amount of fat matched that of the CM as measured for the individual's weight range. Overall, the CM treatment provided an average of 1.9 g CHO, 0.6 g PRO, and 0.3 g fat per kg body weight. The CHO treatment provided an average of 2.5 g CHO and 0.3 g fat per kg body weight.

Diet and exercise. The subjects were instructed to maintain training and dietary logs for the 2 and 3 days, respectively, before the familiarization trial and to keep training and diet consistent with that record for the days prior to the remaining 3 experimental trials. The subjects provided a copy of their training and dietary logs on the day of each trial. An investigator reviewed and verified the entries in the logs with the subjects at each session in order to verify that compliance with the previous logs was attained. The data from the logs were entered into Nutribase Clinical Nutrition Manager 7.17 (CyberSoft, Inc., Phoenix, AZ) for nutritional analysis. Diets were not standardized, as each subject served as his or her own control. All subjects complied with the diet and exercise requirements.

Blood sampling and analyses. Blood sampling occurred at 6 time points during the protocol as shown in Figure 4.1. Saline flushes occurred every 10-15 min during the entire protocol to keep the catheter patent. Each 6 ml blood sample was mixed with 0.5 ml of EDTA (24 mg/mL, pH 7.4), and 0.3 ml of the anticoagulated blood was transferred to another tube containing 0.6 ml 10% perchloric acid (PCA). All tubes were centrifuged at 4˚ C for 10 min at 3,000 rpm with a HS-4 rotor in a Sorvall RC6 centrifuge (Kendro Laboratory Products, Newtown, CT). After centrifugation, plasma and PCA extracts were separated into aliquots for each assay and immediately frozen and stored at -80˚ C for later analysis. For all assays, all samples were run in duplicate. Plasma glucose was measured using a spectophotometric Trinder reaction (no. 315, Sigma Chemical, St.

Louis, MO), and had a coefficient of variation (CV) of 3.7%. Plasma insulin was measured using ImmuChem^{TM} Coated Tube ^{125}I RIA Kit (MP Biomedicals, LLC, Orangeburg, NY) by radioimmunoassay with a CV of 6.0%. Blood lactate was determined from the PCA extract by enzymatic-spectophotometric analysis method based on the oxidation of lactate to pyruvate by nicotinamide adenine dicnucleotide (NAD^+)· (18), and had a CV of 1.5%. Plasma free fatty acids (FFA) were measured using the colorimetric assay procedure of Duncombe (13) but modified by using the extraction reagent of Noma et al. (35) and the copper reagent of Laurell and Tribbling (24). The CV for this assay was 5.3%. Plasma glycerol was measured from the PCA extract according to the protocol of Weiland (55). Plasma myoglobin concentrations were determined by solid phase ELISA (BioCheck, Inc., Foster City, CA), with a CV of 5.4%. Plasma CPK was determined spectrophotometrically using the Creatine Kinase Reagent Kit (Pointe Scientific, Inc., Canton, MI). The CV for this assay was 1.0%. Plasma cortisol was measured using ImmuChem^{TM} Coated Tube ^{125}I RIA Kit (MP Biomedicals, LLC, Orangeburg, NY) by radioimmunoassay. The CV for this assay was 6.1%. Total plasma concentrations of interleukin-1 receptor antagonist (IL-1Ra), interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-10 (IL-10), and tumor necrosis factor alpha (TNFα) were determined using Millipore High Sensitivity Multiplex Human Cytokine Assay kits (Millipore, Billerica, MA) with a Bio-Plex 200 multiplex suspension array system with Luminex xMAP detection technology (Luminex Corp., Austin, TX).

Substrate oxidation. Substrate utilization during the recovery period was estimated from the periodic measurements of RER, VCO_2 and VO_2 (min 30-40, 90-100, 150-160 and 210-220 of the recovery period, as shown in Figure 4.1). Fat and carbohydrate oxidation rates were calculated from the VCO_2 and VO_2 values using the equation of Frayn (15). Energy expenditure ($kcal \cdot min^{-1}$) from carbohydrate and fat was calculated from the oxidation rates by applying the Atwater general conversion factors for CHO and fat (2). Results are shown in Table 4.4.

Heart rate and RPE. Heart rate was recorded using Polar heart rate monitors at the beginning of each exercise bout, and every 30 min during exercise, as well as every 30 min during the recovery phase. Subjective RPE ratings on a Borg-scale (ranging from 6 to 20) were obtained during exercise at the same time points as heart rate during exercise bouts.

Muscle biopsy procedure. Prior to each biopsy**,** the subject's thigh was cleansed with 10% betadine solution and 1.4 mL of 1% Lidocaine Hydrochloride (Elkins-Sinn, Inc., Cherry Hill, NJ) was injected. The first biopsy of each trial (R0) was taken from the vastus lateralis through a 5-8 mm incision made through the skin and fascia, 6 inches from the midline of the thigh on the lateral side and 2.5 inches above the patella. The second biopsy (R45) was taken from the same incision as the R0 sample. A new incision was made for the third biopsy (R End), one inch above the previous incision. The same leg was used for the first and third experimental trials, with the other leg biopsied in the second trial. The incisions for the third trial were made one inch above and proximal to the incisions from the first trial. Approximately ~45-60 mg wet wt of tissue was taken during each biopsy. The tissue samples were trimmed of adipose and connective tissue and immediately frozen in liquid nitrogen at -80^{o} C for subsequent analysis.

Muscle tissue processing. The muscle samples were weighed and homogenized in ice-cold buffer containing 20 mM Hepes, 2 mM EGTA, 50 mM sodium fluoride, 100 mM potassium chloride, 0.2 mM EDTA, 50 mM glycerophosphate, 1 mM DTT, 0.1 mM PMST, 1 mM benzamidine, and 0.5 mM sodium vanadate (pH 7.4) at a dilution of 1:100. Homogenization was performed on ice using Caframo RZRl Stirrer (Caframo Limited, Warton, Ontario, Canada). Half of the homogenate was used immediately for the determination of muscle glycogen concentration. The other half of the homogenate was immediately centrifuged at 14,000 g for 10 min at 4°C and the supernatant aliquoted to several test tubes and stored at -80°C for later signaling protein analysis. Phosphorylation

of mTOR, rpS6, eIF2Bε, and FOXO3A, total ubiquitination, and total α–tubulin was determined by Western blotting.

Muscle glycogen analysis. Muscle glycogen concentrations were determined from the freshly homogenized muscle samples after the complete enzymatic degradation from glycogen to glucose with amyloglucosidase (37). Liberated glucose was then measured using a spectophotometric Trinder reaction (no. 315, Sigma Chemical, St. Louis, MO).

Muscle intracellular signaling protein analyses. Using the supernatant aliquoted for use in signaling protein analysis, protein concentration was determined using a modified Lowry Assay (29). Aliquots of homogenized muscle sample supernatants and standards were slowly thawed over ice and diluted 1:1 with sample buffer containing 1.25M Tris, pH 6.8, glycerol, 20% SDS, 2-mercaptoethanol, 0.25% bromophenol blue solution, and deionized water. 3 gels were electrophoresed for each subject's 9 samples: one for detection of p-mTOR and p-FOXO3A, one for p-eIF2Bε and p-rpS6, and one for ubiquitination. Total α-tubulin, measured as a housekeeping protein, was also detected on each gel.

Samples containing 60 μg of total protein were separated on 10% polyacrylamide gels SDS-PAGE for 48 min (eIF2Bε and rpS6), 104 min (mTOR and FOXO3A), or 45 min (ubiquitnation) at 200 V (Bio-Rad Laboratories, Hercules, CA.) After electrophoresis, the gels were electrotransferred using 25 V for 15 min (eIF2Bε, rpS6 and ubiquitination) or 22 min (mTOR and FOXO3A) to 0.4 μm polyvinylidine fluoride (PVDF) membranes (Millipore, Bedford, MA). The membranes were blocked in TTBS (TBS, 50 mM Tris, 150 mM NaCl, containing 0.06% Tween-20), and 6% nonfat dry milk (or 5% BSA in the case of eIF2Bε) for 1 h at room temperature on a rocking platform at medium speed. The membranes were then washed in 1x TTBS 3 times for 5 min each wash. Using the molecular weight markers visible on the membranes in three lanes as a guide, the membrane for mTOR (~289 kD), FOXO3A (~97 kD), and a-tubulin (~55 kD) was cut into three sections at the 150 and 75 kD markers. The membranes for eIF2Bε (~82 kD),

rpS6 (~32 kD), and a-tubulin (~55 kD) were cut into 3 sections at the 75 and 50 kD markers. The membrane for ubiquitination was not cut. (After probing for ubiquitination, the membrane section was stripped, reblocked in 6% NFDM-TTBS, and reprobed for α-tubulin).

Each membrane or section of membrane was incubated overnight at 4°C on a rocking platform at low speed with antibodies directed against p-mTOR (Ser2448, no. 2971S, Cell Signaling, Danvers, MA), p-rpS6 (Ser235/236, no. 9205S, Cell Signaling, Danvers, MA.), p-eIF2Bε (Ser539, no. PS1017, EMD Calbiotech/Merck KGaA, Darmstadt, Germany), p-FOXO3A (Ser318/321, no. 9465, Cell Signaling, Danvers, MA), ubiquitin (no. 9133, Santa Cruz Biotechnology, Santa Cruz, CA,) and α-tubulin (no. 2144, Cell Signaling, Danvers, MA). The antibodies were diluted 1:800 (mTOR), 1:900 (α-tubulin), 1:500 (FOXO3A and ubiquitin), and 1:1000 (rpS6 and eIF2Bε) in TTBS containing 2% nonfat dry milk for mTOR, α-tubulin, and ubiquitin; for FOXO3A and eIF2Bε, primary incubation was done in TTBS with 5% and 1% BSA (no. 105033, ICN Biochemicals, Costa Mesa, CA), respectively. Following the overnight incubation, membranes were washed 3 times with TTBS for 5 min each wash and incubated for 1.5 h for mTOR, eIF2Bε and FOXO3A, and 2 h for α-tubulin and ubiquitin) with a secondary antibody (goat anti-rabbit, HRP-linked IgG, no. 7074, Cell Signaling, Danvers, MA). Dilutions were 1:800 (mTOR), 1:1000 (α-tubulin), 1:1500 (FOXO3A), and 1:2000 (rpS6) in TTBS containing 2% nonfat dry milk. For eIF2Bε, secondary incubation was in TTBS with 1% BSA (no. 105033, ICN Biochemicals, Costa Mesa, CA.) at a dilution of 1:3000. The antibody-bound proteins on the immunoblots were visualized by enhanced chemiluminescence according to the manufacturer's protocol (Perkin Elmer, Boston, MA) using a Bio-Rad ChemiDoc detection system, and the mean density of each band was quantified using Quantity One 1-D Analysis software (Bio-Rad Laboratories, Hercules, CA.). A molecular weight ladder (Precision Plus Protein Standard, Bio-Rad) and a rodent internal control standard prepared from insulin-stimulated mixed skeletal muscle were also included on each gel. All blots were compared with the rodent control

standard and the values of each sample were represented as a percent of standard for each blot.

Statistical analyses. Plasma insulin, glucose, lactate, myoglobin, CPK, FFA, glycerol, cortisol, and cytokines were analyzed using two-way (treatment x time) analysis of variance (ANOVA) for repeated measures. Time trial measures (TT time, average power output, average heart rate, and average RPE) were analyzed using one-way ANOVA for repeated measures. Differences in muscle glycogen resynthesis from R0 to R45, R45 to R End and Total resynthesis (R0 to R End) was analyzed using a two-way (treatment x time) ANOVA for repeated measures. Signaling protein phosphorylation or content was analyzed using two-way ANOVAs for repeated measures (treatment x time and time x treatment). Substrate oxidation and energy expenditure during the recovery period were analyzed using two-way (treatment x time) ANOVA for repeated measures. For all measures, post hoc analysis was performed when significance was found using Least Significant Difference (LSD). Differences were considered significant at $P<0.05$. Data were expressed as mean ± SE. All statistical analyses were performed using SPSS version 16.0 statistical software (SPSS Inc., Chicago, IL).

RESULTS

Time trial performance. Time trial time (TTT) is shown in Figure 4.2A. TTT was significantly shorter in CM compared to both CHO and PLA ($P<0.05$). Average power output (Watts) was significantly higher in CM compared to both CHO and PLA (Figure 4.2B; $P<0.05$). Heart rate during the TT was significantly higher in CM than in CHO or PLA (162.8 ± 5.6 vs 145.9 ± 7.4 and 148.9 ± 7.1, respectively, $P<0.05$), while RPE was not significantly different between the 3 treatments (14.5 ± 0.5 vs 14.0 ± 0.5 and 14.0 ± 0.5, respectively, $P<0.05$).

Muscle glycogen. Muscle glycogen resynthesis values are shown in Figure 4.3. Total muscle glycogen resynthesis over the 4 h recovery period was significantly greater in

both the CM and CHO treatments than PLA (23.58 and 30.58 μmol/g wet wt, respectively, vs 7.05 μmol/g wet wt, P <0.05). CHO was not significantly different from CM, although significance was approached (P = 0.06).

Blood and plasma analyses. A significant treatment effect existed for plasma glucose in CHO compared to CM and PLA, and in CM compared to PLA (Figure 4.4A; P <0.05). At R45, plasma glucose in CHO was significantly higher than CM and PLA, and CM was significantly higher than PLA (P <0.05). At R120, both CHO and CM were higher than PLA (P <0.05), with no significant differences between CHO and CM. At R End and TT End, no differences existed between the 3 treatments (Figure 4.4A). Plasma insulin (Figure 4.4B) was also significantly higher in the CHO treatment compared to CM and PLA, and CM compared to PLA (P <0.05). Both CHO and CM were significantly higher than PLA at R45 (P <0.05). At R120 and R End, plasma insulin levels in CM had decreased to near baseline levels but were still significantly higher than PLA (P <0.05). However, plasma insulin in CHO was significantly higher at R120 and R End than CM and PLA. At TT End, no treatment differences were detected (Figure 4.4B; P <0.05).

A significant overall treatment effect for blood lactate was observed between CM and PLA (P <0.05). As shown in Figure 4.5, blood lactate levels rose significantly during the first exercise bout (Pre to R0) in all treatment groups (P <0.05), although a slight but significantly lower blood lactate level was found in the PLA treatment at R0 and R45 compared to CM (P <0.05). No differences existed between the 3 treatments at R120 and R End. At TT End, blood lactate was significantly higher in CM and CHO than PLA (P <0.05). For both plasma glycerol and free fatty acids (FFAs), significant treatment effects were found in PLA compared to CHO and CM (P <0.05), and between CM and CHO (Figure 4.6A and B; P <0.05). At R45, plasma glycerol was significantly higher in PLA than CM only, and FFAs were significantly higher in PLA than both CM and CHO (P <0.05). At R120 and R End, plasma glycerol and FFAs were significantly higher in PLA than CHO and CM, and in CM compared to CHO (P <0.05). At TT End, plasma glycerol

in CHO was significantly lower than CM and PLA (P <0.05), and plasma FFAs were higher in PLA compared to both CHO and CM at TT End (P <0.05), suggesting that more fat was available to be used as fuel in the PLA and CM treatments compared to CHO.

As is shown in Table 4.3, plasma myoglobin, CPK, cortisol, and cytokines IL-6, IL-8, IL-1Ra, and TNFα rose significantly over time in all treatments (P <0.05), with no significant differences found between the treatments. The increase in all of these analytes from Pre to R0, as well as in blood lactate, indicate that the subjects were cycling at a high intensity during the initial bout. All decreased from the R0 values during the 4-h recovery period, and increased at TT End, with no significant treatment differences detected.

Intracellular signaling proteins. Significant differences in mTOR phosphorylation are shown in Figure 4.7. A significant overall treatment difference was found for mTOR phosphorylation between CM and PLA (P<0.05). No difference in phosphorylation status of mTOR was detected between treatments at R0. However, at 45 min of recovery (R45), mTOR phosphorylation was significantly greater in the CM treatment compared to the CHO and PLA treatments (Figure 4.7). At the end of recovery (R End), phosphorylation was greater in CM compared to PLA (P<0.05). At R45, phosphorylation in both CM and CHO was significantly greater than at R0 for the respective treatments, and at R End, this elevated phosphorylation status persisted only in CM (Figure 4.7; P<0.05).

The phosphorylation pattern of rpS6 (Figure 4.8) was similar to that of mTOR, with a significant overall treatment difference between CM vs CHO and PLA (P<0.05). At R0, no differences in rpS6 phosphorylation were detected between treatments. At R45, phosphorylation of rpS6 was increased significantly, with the increase in CM significantly greater than CHO and PLA (P<0.05). At R End, phosphorylation in CM remained elevated compared to the R0 value, and was significantly greater than CHO and

PLA (P<0.05), while phosphorylation in CHO and PLA had returned to levels that were not significantly greater than their respective R0 values (Figure 4.8).

FOXO3A phosphorylation is shown in Figure 4.9. A significant overall treatment difference existed for FOXO3A phosphorylation between CHO and PLA (P<0.05). At R0, no differences in FOXO3A phosphorylation existed between treatments. At R45, FOXO3A phosphorylation was significantly greater in CHO and CM compared to PLA, as well as compared to the respective R0 levels (all P<0.05). By R End, phosphorylation remained elevated only in CHO compared to CM and PLA, and compared to the R0 level (Figure 4.9, P<0.05).

No differences were detected for p-eIF2Bε (Figure 4.10), total ubiquitination (Figure 4.11A), or percent change in ubiquitination (Figure 4.11B) for treatment, time or treatment x time interactions.

Substrate utilization during recovery. Carbohydrate and fat energy expenditure, and carbohydrate and fat oxidation rates during the recovery period are shown in Table 4.4. Average carbohydrate energy expenditure (kcal·min^{-1}) and carbohydrate oxidation (g·min^{-1}) was significantly lower in PLA than CM or CHO (P<0.05). Average fat energy expenditure (kcal·min^{-1}) and fat oxidation (g·min^{-1}) was significantly lower in the CHO treatment than CM or PLA (both P<0.05).

DISCUSSION

The present study aimed to characterize the roles of an initial bout of strenuous exercise and nutritional supplementation on subsequent exercise performance and key aspects of exercise recovery, namely muscle glycogen resynthesis, markers of muscle damage and inflammation, and activation states of intramuscular signaling proteins involved in protein synthesis and degradation. The key finding of the present study is that a popular dairy beverage containing both carbohydrate and protein (low fat chocolate milk, CM),

was more effective in improving cycling time trial performance in a subsequent bout compared to an isocaloric carbohydrate or a placebo supplement. This performance improvement occurred despite there being no increase in the subjective ratings of perceived exertion (RPE), suggesting that they did not perceive their effort as more difficult although they were able to exercise at a higher intensity with the CM treatment compared to CHO and PLA. Furthermore, we report here that CM increased the activation status of signaling proteins associated with increased mRNA translation and protein synthesis compared to placebo. These findings are novel because the recovery supplement used in comparison to CHO and placebo is a commonly available, organic dairy beverage, CM, rather than a CHO+PRO supplement commercially designed for exercise recovery. In addition, the present investigation examines many aspects of the recovery process with the aim of addressing possible mechanisms of improved recovery and performance.

The finding of improved subsequent performance is in agreement with several other studies that reported improved endurance performance in a subsequent bout when ingesting a CHO+PRO supplement compared to CHO alone (3, 10, 34, 45, 56), as well as with those of investigations comparing CM to other CHO+PRO supplements (39) and to both CHO+PRO and CHO only supplements (49). However, not all investigations report improvements in subsequent performance with CHO+PRO supplementation compared to CHO (7, 8, 42-44). Differences in protocol design may lead to the inconsistent findings across investigations of CHO+PRO (including CM) supplementation. In some studies, supplements were given during the initial exercise bout (7,8) or both during and post-exercise (45), while in the present study and others, the supplements were given immediately post-exercise and again during the recovery period (34, 56). The timing of the subsequent exercise trials also varied between studies, including ~15 h later (45, 39), 22-24 h later (7, 8, 42-44, 36) 1 and 2 days following the initial bout (44) or following a 3-4 h recovery, as in the current study (39, 49, 56). Differences also exist for the type of exercise trial employed to assess subsequent performance and recovery: time trial time

(7, 8), time to exhaustion (36, 42, 43, 45), or repeated sprint performance (44). Therefore, these and other methodological differences must be considered when interpreting results of CHO+PRO vs CHO investigations.

One proposed mechanism for improved performance following recovery with CHO+PRO supplementation is increased muscle glycogen resynthesis during the recovery period. Several investigations have shown that CHO+PRO supplementation can increase the rate of muscle glycogen synthesis beyond that of CHO alone (4, 21, 51, 56, 58), and therefore may contribute to improved exercise performance in a subsequent bout. In the present study, we did not find a significant difference between CM and CHO in muscle glycogen resynthesis, although both were significantly greater than PLA. However, only in the CM treatment was TT performance significantly improved compared to CHO and PLA. Interestingly, although CHO demonstrated a slight, non-significantly higher ($P<0.06$) total muscle glycogen resynthesis compared to CM, this did not result in improved TT performance in CHO compared to PLA. Therefore, the data suggest that muscle glycogen synthesis is not the mechanism through which subsequent performance was improved in the current study. Possible reasons for the lack of a relationship between TT performance and muscle glycogen resynthesis are that muscle and liver glycogen levels were perhaps not sufficiently depleted in the initial bout, and the length of the subsequent bout (40 km) may not have been long enough for muscle glycogen to be a rate-limiting factor in performance.

The lack of a significant difference in muscle glycogen resynthesis between CM and CHO may be due to the observed difference in the insulin response. Although we found a lower plasma insulin response with CM compared to CHO in the current study, previous investigations have shown higher insulin responses with CHO+PRO compared to CHO (47, 56, 58). However, most prior investigations of CHO+PRO supplementation have used a whey protein-containing CHO+PRO beverage rather than CM. It is likely that the different osmolarity and gastric emptying rate of CM compared to CHO could have

slowed the intestinal transport of carbohydrates and amino acids, resulting in a lower plasma glucose and insulin response, as well as their subsequent availability to the muscle, in CM compared to CHO. This likely explains the lack of a significant difference in muscle glycogen resynthesis between these two nutrient-containing treatments. In addition, CM contains many micronutrients that may not be found in other CHO+PRO supplements, and the effects of these components on the measures investigated here are unknown at this time.

Another proposed mechanism for subsequent performance improvement with a CHO+PRO-containing supplement is muscle damage attenuation. Saunders and colleagues (45) demonstrated that CHO+PRO supplementation improved recovery from an initial strenuous exercise bout such that subsequent exercise performance was improved, and noted that muscle damage marker creatine phosphokinase (CPK) was suppressed in the CHO+PRO treatment. In the current study, however, this association between improved subsequent performance and reduced muscle damage indicators myoglobin and CPK was not demonstrated. In addition, we did not detect treatment differences in any of the pro- or anti-inflammatory cytokines (Table 4.3). Taken together, our findings suggest that neither reduced muscle damage nor a reduced inflammatory response during the recovery period underlies the subsequent performance differences 4 h later.

In addition to plasma markers of muscle damage and inflammation, we assessed two proteins in the skeletal muscle samples that regulate protein degradation—FOXO3A and ubiquitin. Protein degradation involves the Ubiquitin-Proteasome Pathway (UPP), the primary degradation system for skeletal muscle (25, 48). A key component of the UPP is ubiquitin itself. This highly conserved, 8.5 kD protein covalently binds to abnormal or damaged proteins, targeting them to be degraded by the 26S proteasome (9, 17). Recently, a few investigations have used total ubiquitin content of Western blot samples as an indicator of protein degradation, demonstrating an increase in total ubiquitination in

conditions of high proteolysis (14, 38). This method was employed in the current study to assess total ubiquitination in the biopsy samples during the recovery period, and despite a trend for slightly increased ubiquitination in the CHO treatment, there was not a statistically significant difference in treatment or in time between the three treatments (Figure 4.11).

Key apoptotic genes in the UPP share a common critical transcription factor, forkhead box 3A (FOXO3A). When the insulin signaling pathway is activated, Akt is phosphorylated, translocates into the nucleus, and phosphorylates FOXO3A on serine residues including 253, 318 and 321, which causes the nuclear export of FOXO3A into the cytoplasm (19, 33, 46). Thus, FOXO3A is deactivated and prevented from promoting apoptosis. A few studies have verified a role for FOXO3A in proteolysis following resistance exercise (26, 28, 41, 57), and running exercise (28). In the present study, we hypothesized that we would detect greater FOXO3A phosphorylation in the CM treatment compared to CHO and PLA. FOXO3A phosphorylation increased significantly from R0 to R45 in both CM and CHO (Figure 4.10, $P<0.05$), and while phosphorylation in both CM and CHO was significantly greater than in PLA, no difference was found between CM and CHO. At R End, FOXO3A phosphorylation remained elevated from R0 only in CHO, and was significantly greater compared to PLA. Since FOXO3A is activated through the insulin signaling pathway, the increased phosphorylation of FOXO3A at R45 in CHO and CM and at R End in CHO likely demonstrates the effect of insulin. Since the insulin response in CHO at R End was higher than in CM or PLA, it appears that this was responsible for the greater FOXO3A phosphorylation at R End in CHO compared to PLA. However, it also appears that the small increase in phosphorylation in FOXO3A was not sufficient to limit protein degradation in the CM and CHO treatments as verified by the markers of muscle damage assessed, and the total ubiquitination results.

Our finding that degradation signaling was not significantly reduced with supplementation in the present study is supported by the lack of reductions in the plasma muscle damage and inflammatory markers. Given that the CM and CHO treatments did not appear to block the processes of muscle damage and degradation, it may be that providing supplementation after the initial exercise bout, rather than both during and post-exercise as has been done previously (45), minimized the effect of supplementation in these areas. Therefore, these data suggest that while attenuation of muscle damage or decreased protein degradation during recovery from strenuous exercise may occur in response to CHO+PRO/CM supplementation, these are not the mechanisms by which TT performance was improved in the current study.

Activation of protein synthesis is an important metabolic response in the recovery process, and is paramount for muscle tissue repair and training adaptation. As mentioned previously, the balance between protein degradation and synthesis post-exercise is mediated in part by the availability of nutrients, most importantly, the availability of AAs. AA infusion or ingestion has been shown to stimulate muscle protein synthesis both at rest (5, 13) and after resistance exercise (6, 50), as has the combination of essential AA and CHO following resistance exercise (11, 40). There is increasing evidence that the combined ingestion of CHO and either protein or AA post-exercise can have an additive effect on protein synthesis (27, 31). This is likely due in part to the synergistic effect of CHO/AA or CHO+PRO supplementation on the plasma insulin response (47, 58), and the maintenance of an elevated plasma AA profile. This may lead to a greater activation of translation initiation. As the rate-limiting step in mRNA translation, translation initiation is regulated in part by the mTOR pathway. mTOR phosphorylates two downstream targets, including p70S6K (30). p70S6K then phosphorylates ribosomal protein S6 (rpS6), which enhances translation of a class of mRNAs that encode ribosomal proteins, elongation factors, and binding proteins (23). This results in an increase in the capacity of the cell to synthesize protein. The eukaryotic initiation factor 2B (eIF2B) also plays a key role in translation initiation. eIF2B catalyzes

the GDP/GTP exchange on eIF2, which is essential for control of ribosomal formation and mRNA translation (22). eIF2Bε is the largest catalytic subunit of this protein and is phosphorylated by GSK-3B at Ser 539 (53), which inhibits the guanine nucleotide exchange activity of eIF2B. Phosphorylation of GSK-3B via the insulin signaling pathway inhibits GSK-3B, releasing its inhibition of eIF2Bε.

In the present study, mTOR phosphorylation increased significantly from R0 to R45 only in CM and CHO, and mTOR phosphorylation in CM was significantly greater than in CHO or PLA. At R End, mTOR phosphorylation in both CM and CHO was still significantly greater than in PLA, which remained unchanged throughout recovery. Only in CM was mTOR phosphorylation still significantly elevated compared to R0. rpS6 phosphorylation increased from R0 to R45 in all treatments, but only CM was significantly higher than PLA. At R End, rpS6 phosphorylation in CM remained elevated compared to R0, and the treatment difference between CM and PLA remained significant. The increased phosphorylation of mTOR and rpS6 in CM compared to CHO and PLA at R45 is likely due to the combined effects of AA and insulin, while the increase in CHO over PLA is likely due to the effect of insulin.

Based on previous results from our laboratory that demonstrated significantly increased phosphorylation of GSK-3B with CHO+PRO supplementation post-endurance exercise compared to exercise only (20), we hypothesized that we would demonstrate reduced phosphorylation of eIF2Bε, a downstream target of GSK-3B, with the CM treatment compared to CHO and PLA. However, we did not detect a significant difference in treatment or time for eIF2Bε phosphorylation in the present study. This finding is supported by that of Glover and colleagues (16), who found that while eIF2Bε phosphorylation was significantly decreased by a strenuous resistance exercise bout compared to a baseline measure, post-exercise supplementation containing CHO, PRO and fat had no additional effect during a 6-h recovery (16). Despite demonstrating increased activation of some of the key components of the mTOR pathway in the present

study, it may be that eIF2Bε phosphorylation may not be responsive to nutrient intervention, since eIF2B can be activated independent of the mTOR pathway (54). Therefore, it is possible that the effects of exercise alone are sufficient for activation of this enzyme, as demonstrated by Glover and colleagues (16).

The greater phosphorylation status of many of the key proteins involved in mRNA translation initiation and protein synthesis in the present investigation is in agreement with previous findings from our laboratory. Recently, Ivy and colleagues (20) investigated the effects of a CHO+PRO supplement compared to placebo on intracellular signaling proteins involved in increased protein and glycogen synthesis. Trained cyclists cycled at 75% VO_2max for 45 min, followed by 5 one-min sprints alternating between 90% and 45% VO_2max. Immediately post-exercise and again 15 min later, subjects ingested 400 ml of a CHO+PRO beverages (7.8% dextrose and 1.8% protein-electrolyte) or placebo. Biopsies of the vastus lateralis were taken before exercise and at 45 min of recovery. At 45 min, phosphorylation of mTOR and rpS6 were increased above pre-exercise levels in the CHO+PRO treatment compared to placebo (20). The results of the present study extend these previous findings by comparing a CHO+PRO supplement, CM, to an isocaloric CHO as well as to placebo. Taken together, these results suggest that while providing nutritional supplementation post-exercise is more effective in increasing the stimulus for protein synthesis and reducing protein degradation than when post-exercise nutritional intake is absent, a CHO+PRO-containing supplement such as CM is more effective overall than CHO alone in promoting an anabolic intracellular environment post-exercise.

In conclusion, CM is more effective than isocaloric carbohydrate or placebo in improving subsequent time trial performance. CM is also more effective in modulating the activation of key intracellular signaling proteins involved in protein synthesis during recovery from endurance exercise. This greater stimulus for protein synthesis, in addition to the possible attenuation of muscle protein degradation and faster tissue repair, during

recovery may be advantageous under conditions when rapid recovery between race events or training sessions is necessary. CM supplementation may also lead to increased training adaptations over time if the exercise and supplementation is performed on a regular basis. More research is necessary to determine the effects of CM in other models of exercise recovery, such as after resistance exercise.

REFERENCES

1. Åstrand PO, and Rodahl K. Textbook of work physiology: Physiological bases of exercise. . New York: McGraw-Hill Book Company, 1977.
2. Atwater W. Coefficients of digestibility and availability of the nutrients of food. Proceedings of the American Physiology Society 30: 14-19, 1909.
3. Berardi JM, Noreen EE, and Lemon PW. Recovery from a cycling time trial is enhanced with carbohydrate-protein supplementation vs. isoenergetic carbohydrate supplementation. J Int Soc Sports Nutr 5: 24, 2008.
4. Berardi JM, Price TB, Noreen EE, and Lemon PW. Postexercise muscle glycogen recovery enhanced with a carbohydrate-protein supplement. Med Sci Sports Exerc 38: 1106-1113, 2006.
5. Biolo G, Tipton KD, Klein S, and Wolfe RR. An abundant supply of amino acids enhances the metabolic effect of exercise on muscle protein. Am J Physiol Endocrinol Metab 273: E122-129, 1997.
6. Borsheim E, Tipton KD, Wolf SE, and Wolfe RR. Essential amino acids and muscle protein recovery from resistance exercise. Am J Physiol Endocrinol Metab 283: E648-657, 2002.
7. Breen L, Tipton KD, and Jeukendrup AE. No effect of carbohydrate-protein on cycling performance and indices of recovery. Med Sci Sports Exerc 42: 1140-1148, 2010.
8. Cermak NM, Solheim AS, Gardner MS, Tarnopolsky MA, and Gibala MJ. Muscle metabolism during exercise with carbohydrate or protein-carbohydrate ingestion. Med Sci Sports Exerc 41: 2158-2164, 2009.
9. Ciechanover A. The ubiquitin-proteasome proteolytic pathway. Cell 79: 13-21, 1994.
10. Combest T, Saunders M, Kane M, and Todd K. Attenuated CPK Following Carbohydrate/protein Intervention Improves Subsequent Performance. Med Sci Sports Exc 37: S42, 2005.
11. Dreyer HC, Drummond MJ, Pennings B, Fujita S, Glynn EL, Chinkes DL, Dhanani S, Volpi E, and Rasmussen BB. Leucine-enriched essential amino acid and carbohydrate ingestion following resistance exercise enhances mTOR signaling and protein synthesis in human muscle. Am J Physiol Endocrinol Metab 294: E392-400, 2008.
12. Drummond MJ, Glynn EL, Fry CS, Dhanani S, Volpi E, and Rasmussen BB. Essential Amino Acids Increase MicroRNA-499, -208b, and -23a and Downregulate Myostatin and Myocyte Enhancer Factor 2C mRNA Expression in Human Skeletal Muscle. J Nutr 139: 2279-2284, 2009.
13. Duncombe WG. The colorimetric micro-determination of non-esterified fatty acids in plasma. Clin Chim Acta 9: 122-125, 1964.
14. Dupont-Versteegden EE, Fluckey JD, Knox M, Gaddy D, and Peterson CA. Effect of flywheel-based resistance exercise on processes contributing to muscle atrophy during unloading in adult rats. J Appl Physiol 101: 202-212, 2006.

15. Frayn KN. Calculation of substrate oxidation rates in vivo from gaseous exchange. J Appl Physiol 55: 628-634, 1983.
16. Glover EI, Oates BR, Tang JE, Moore DR, Tarnopolsky MA, and Phillips SM. Resistance exercise decreases eIF2B{varepsilon} phosphorylation and potentiates the feeding-induced stimulation of p70S6K1 and rpS6 in young men. Am J Physiol Regulatory Integrative Comp Physiol 295: R604-610, 2008.
17. Hochstrasser M. Ubiquitin, proteasomes, and the regulation of intracellular protein degradation. Curr Opin Cell Biol 7: 215-223, 1995.
18. Hohorst HJ. Determination of L-lactate with LDH and DPN. In: Methods of Enzymatic Analysis, edited by Bergmeyer HU. New York: Academic, 1963, p. 266-270.
19. Huang H, and Tindall DJ. Dynamic FoxO transcription factors. J Cell Sci 120: 2479-2487, 2007.
20. Ivy JL, Ding Z, Hwang H, Cialdella-Kam LC, and Morrison PJ. Post exercise carbohydrate-protein supplementation: phosphorylation of muscle proteins involved in glycogen synthesis and protein translation. Amino Acids 35: 89-97, 2008.
21. Ivy JL, Goforth HW, Jr., Damon BM, McCauley TR, Parsons EC, and Price TB. Early postexercise muscle glycogen recovery is enhanced with a carbohydrate-protein supplement. J Appl Physiol 93: 1337-1344, 2002.
22. Kimball SR, Farrell PA, and Jefferson LS. Exercise Effects on Muscle Insulin Signaling and Action: Invited Review: Role of insulin in translational control of protein synthesis in skeletal muscle by amino acids or exercise. J Appl Physiol 93: 1168-1180, 2002.
23. Kimball SR, and Jefferson LS. Regulation of global and specific mRNA translation by oral administration of branched-chain amino acids. Biochem Biophys Res Commun 313: 423-427, 2004.
24. Laurell S, and Tibbling G. Colorimetric micro-determination of free fatty acids in plasma. Clin Chim Acta 16: 57-62, 1967.
25. Lecker SH, Goldberg AL, and Mitch WE. Protein Degradation by the Ubiquitin-Proteasome Pathway in Normal and Disease States. J Am Soc Nephrol 17: 1807-1819, 2006.
26. Leger B, Cartoni R, Praz M, Lamon S, Deriaz O, Crettenand A, Gobelet C, Rohmer P, Konzelmann M, Luthi F, and Russell AP. Akt signalling through GSK-3{beta}, mTOR and Foxo1 is involved in human skeletal muscle hypertrophy and atrophy. J Physiol 576: 923-933, 2006.
27. Levenhagen DK, Carr C, Carlson MG, Maron DJ, Borel MJ, and Flakoll PJ. Postexercise protein intake enhances whole-body and leg protein accretion in humans. Med Sci Sports Exerc 34: 828-837, 2002.
28. Louis E, Raue U, Yang Y, Jemiolo B, and Trappe S. Time course of proteolytic, cytokine, and myostatin gene expression after acute exercise in human skeletal muscle. J Appl Physiol 103: 1744-1751, 2007.
29. Lowry OH, Rosebrough NJ, Farr AL, and Randall RJ. Protein measurement with the folin phenol reagent. J Biol Chem 193: 265-275, 1951.

30. Martin DE, and Hall MN. The expanding TOR signaling network. Current Opinion in Cell Biology 17: 158-166, 2005.
31. Miller SL, Tipton KD, Chinkes DL, Wolf SE, and Wolfe RR. Independent and combined effects of amino acids and glucose after resistance exercise. Med Sci Sports Exerc 35: 449-455, 2003.
32. Morrison PJ, Hara D, Ding Z, and Ivy JL. Adding protein to a carbohydrate supplement provided after endurance exercise enhances 4E-BP1 and RPS6 signaling in skeletal muscle. J Appl Physiol 104: 1029-1036, 2008.
33. Nakae J, Oki M, and Cao Y. The FoxO transcription factors and metabolic regulation. FEBS Lett 582: 54-67, 2008.
34. Niles ES, Lachowetz T, Garfi J, Sullivan W, Smith JC, Leyh BP, and Headley SA. Carbohydrate-Protein Drink Improves Time to Exhaustion after Recovery from Endurance Exercise. JEPonline 4: 45-52, 2001.
35. Noma A, Okabe H, and Kita M. A new colorimetric micro-determination of free fatty acids in serum. Clin Chim Acta 43: 317-320, 1973.
36. Osterberg KL, Zachwieja JJ, and Smith JW. Carbohydrate and carbohydrate+protein for cycling time-trial performance. Journal of Sports Sciences 26: 227 - 233, 2008.
37. Passonneau JV, and Lauderdale VR. A comparison of three methods of glycogen measurement in tissues. Anal Biochem 60: 405-412, 1974.
38. Pierce AP, de Waal E, McManus LM, Shireman PK, and Chaudhuri AR. Oxidation and structural perturbation of redox-sensitive enzymes in injured skeletal muscle. Free Radic Biol Med 43: 1584-1593, 2007.
39. Pritchett K, Bishop P, Pritchett R, Green M, and Katica C. Acute effects of chocolate milk and a commercial recovery beverage on postexercise recovery indices and endurance cycling performance. Appl Physiol Nutr Metab 34: 1017-1022, 2009.
40. Rasmussen BB, Tipton KD, Miller SL, Wolf SE, and Wolfe RR. An oral essential amino acid-carbohydrate supplement enhances muscle protein anabolism after resistance exercise. J Appl Physiol 88: 386-392, 2000.
41. Raue U, Slivka D, Jemiolo B, Hollon C, and Trappe S. Proteolytic Gene Expression Differs At Rest and After Resistance Exercise Between Young and Old Women. J Gerontol A Biol Sci Med Sci 62: 1407-1412, 2007.
42. Romano B, Todd M, and Saunders M. Effect of a 4: 1 Ratio Carbohydrate/Protein Beverage on Endurance Performance, Muscle Damage and Recovery. Med Sci Sports Exc 36: S126, 2004.
43. Romano-Ely B, Todd M, Saunders M, and St. Laurent T. Effect of an Isocaloric Carbohydrate-Protein-Antioxidant Drink on Cycling Performance. Med Sci Sports Exc 38: 1608-1616, 2006.
44. Rowlands DS, Thorp RM, Rossler K, Graham DF, and Rockell MJ. Effect of protein-rich feeding on recovery after intense exercise. Int J Sport Nutr Exerc Metab 17: 521-543, 2007.

45. Saunders M, Kane M, and Todd K. Effects of a Carbohydrate-Protein Beverage on Cycling Endurance and Muscle Damage. Med Sci Sports Exc 36: 1233-1238, 2004.
46. Singh A, Ye M, Bucur O, Zhu S, Tanya Santos M, Rabinovitz I, Wei W, Gao D, Hahn WC, and Khosravi-Far R. Protein Phosphatase 2A Reactivates FOXO3a through a Dynamic Interplay with 14-3-3 and AKT. Mol Biol Cell 21: 1140-1152, 2010.
47. Spiller GA, Jensen CD, Pattison TS, Chuck CS, Whittam JH, and Scala J. Effect of protein dose on serum glucose and insulin response to sugars. Am J Clinical Nutrition 46: 474-480, 1987.
48. Taillandier D, Aurousseau E, Meynial-Denis D, Bechet D, Ferrara M, Cottin P, Ducastaing A, Bigard X, Guezennec CY, and Schmid HP. Coordinate activation of lysosomal, Ca 2+-activated and ATP-ubiquitin-dependent proteinases in the unweighted rat soleus muscle. Biochem J 316 (Pt 1): 65-72, 1996.
49. Thomas K, Morris P, and Stevenson E. Improved endurance capacity following chocolate milk consumption compared with 2 commercially available sport drinks. Appl Physiol Nutr Metab 34: 78-82, 2009.
50. Tipton KD, Ferrando AA, Phillips SM, Doyle D, Jr., and Wolfe RR. Postexercise net protein synthesis in human muscle from orally administered amino acids. Am J Physiol Endocrinol Metab 276: E628-634, 1999.
51. van Loon LJC, Saris WHM, Kruijshoop M, and Wagenmakers AJM. Maximizing postexercise muscle glycogen synthesis: carbohydrate supplementation and the application of amino acid or protein hydrolysate mixtures. Am J Clinical Nutrition 72: 106-111, 2000.
52. Wang L, Rhodes CJ, and Lawrence JC, Jr. Activation of Mammalian Target of Rapamycin (mTOR) by Insulin Is Associated with Stimulation of 4EBP1 Binding to Dimeric mTOR Complex 1. J Biol Chem 281: 24293-24303, 2006.
53. Wang X, Paulin FE, Campbell LE, Gomez E, O'Brien K, Morrice N, and Proud CG. Eukaryotic initiation factor 2B: identification of multiple phosphorylation sites in the epsilon-subunit and their functions in vivo. EMBO J 20: 4349-4359, 2001.
54. Weiland O. Glycerol, UV-method. In: Methods of enzymatic analysis, edited by Bergmeyer HU. New York: Academic Press, 1974, p. 1404–1409.
55. Williams MB, Raven PB, Fogt DL, and Ivy JL. Effects of Recovery Beverages on Glycogen Restoration and Endurance Exercise Performance. Journal of Strength and Conditioning Research 17: 12-19, 2003.
56. Williamson DL, Raue U, Slivka DR, and Trappe S. Resistance Exercise, Skeletal Muscle FOXO3A, and 85-Year-Old Women. J Gerontol A Biol Sci Med Sci 65A: 335-343, 2010.
57. Zawadzki KM, Yaspelkis BB, 3rd, and Ivy JL. Carbohydrate-protein complex increases the rate of muscle glycogen storage after exercise. J Appl Physiol 72: 1854-1859, 1992.

	Mean (10)	Males (5)	Females (5)
Age (y)	31.8 ± 1.6	32.2 ± 2.4	31.4 ± 2.5
Mass (kg)	67.8 ± 2.6	72.5 ± 3.1	63.1 ± 3.2
Height (cm)	171.1 ± 3.4	176.8 ± 3.1	165.4 ± 5.1
VO_2max ($L \cdot min^{-1}$)	3.6 ± 0.2	4.2 ± 0.2	3.0 ± 0.1
VO_2max ($ml \cdot kg \cdot min^{-1}$)	52.6 ± 2.3	57.7 ± 2.8	47.6 ± 1.5

Values are mean ± SE.

Table 4.1. Subject characteristics

	CM	CHO	PLA
CHO, g/100 ml	11.48	15.15	0
PRO, g/100 ml	3.67	0	0
Fat, g/100 ml	2.05	2.05	0
kcals/100 ml	79.05	79.05	0
Ratio of CHO:PRO	3.12:1	--	--

Per 100 ml. CM, chocolate milk; CHO, carbohydrate + fat; PLA, placebo.

Table 4.2. Energy and macronutrient content of supplements

	Pre	R0	R45	R120	R End	TT End
Myoglobin (nmol/L)						
CM	1.26 ± 0.11	1.44 ± 0.06	2.16 ± 0.32	--	1.53 ± 0.16	1.87 ± 0.14 [g]
CHO	1.26 ± 0.12	1.45 ± 0.11	2.01 ± 0.36	--	1.91 ± 0.35	2.16 ± 0.32 [g]
PLA	1.35 ± 0.13	1.68 ± 0.20	2.37 ± 0.42	--	1.73 ± 0.10	1.99 ± 0.21 [g]
CPK (U/L)						
CM	125.80 ± 27.42	157.97 ± 38.30	--	146.69 ± 46.22	161.85 ± 45.96	206.03 ± 64.97 [g]
CHO	94.56 ± 22.85	114.34 ± 25.81	--	107.50 ± 23.20	124.78 ± 35.97	167.19 ± 35.53 [g]
PLA	179.25 ± 76.91	207.82 ± 86.59	--	178.79 ± 62.46	207.69 ± 69.82	240.39 ± 69.75 [g]
Cortisol (pmol/L)						
CM	729.1 ± 96.7	837.4 ± 62.1	595.3 ± 61.0	433.9 ± 69.7	382.8 ± 57.5	902.1 ± 69.1 [g]
CHO	698.9 ± 70.0	787.4 ± 119.7	668.4 ± 82.8	539.2 ± 80.1	414.7 ± 53.9	1154.8 ± 143.0 [g]
PLA	720.2 ± 84.2	894.5 ± 133.3	606.3 ± 73.0	491.9 ± 75.4	370.0 ± 44.8	874.7 ± 103.3 [g]
IL-6 (pg/mL)						
CM	38.48 ± 17.01	62.18 ± 21.29	40.24 ± 15.95	57.32 ± 18.38	47.16 ± 15.24	97.39 ± 33.02 [g]
CHO	55.00 ± 20.14	93.01 ± 38.17	58.49 ± 19.23	79.93 ± 23.39	67.01 ± 19.53	97.16 ± 38.56 [g]
PLA	60.17 ± 31.19	109.79 ± 52.59	85.01 ± 38.69	81.86 ± 22.47	81.00 ± 27.10	139.72 ± 57.34 [g]
IL-8 (pg/mL)						
CM	24.51 ± 4.73	38.38 ± 8.26	28.69 ± 5.35	30.35 ± 5.84	26.69 ± 5.21	39.02 ± 8.63 [g]
CHO	29.12 ± 7.18	42.22 ± 9.86	31.65 ± 7.31	29.50 ± 6.32	24.97 ± 5.29	40.82 ± 7.37 [g]
PLA	29.16 ± 6.79	41.68 ± 9.90	37.64 ± 9.04	34.76 ± 8.34	34.21 ± 8.53	40.52 ± 17.14 [g]
IL-10 (pg/mL)						
CM	133.78 ± 87.28	213.63 ± 114.98	174.48 ± 99.87	157.20 ± 78.88	140.04 ± 71.90	286.63 ± 135.93
CHO	124.18 ± 59.86	258.56 ± 130.82	225.11 ± 116.35	237.41 ± 119.95	187.66 ± 86.92	219.92 ± 92.27
PLA	162.30 ± 101.89	264.18 ± 159.08	188.65 ± 88.52	229.49 ± 115.90	206.59 ± 111.96	367.82 ± 210.10
IL-1Ra (pg/mL)						
CM	67.45 ± 8.64	86.19 ± 10.07	67.21 ± 7.16	45.38 ± 11.17	55.27 ± 9.40	54.31 ± 9.74 [g]
CHO	69.83 ± 12.84	78.54 ± 10.49	66.38 ± 8.76	36.32 ± 6.41	47.97 ± 8.72	56.33 ± 7.07 [g]
PLA	65.31 ± 7.60	79.11 ± 6.55	59.52 ± 9.09	49.93 ± 6.29	63.66 ± 7.54	58.15 ± 8.90 [g]
TNFα (pg/mL)						
CM	8.21 ± 1.86	9.23 ± 1.64	6.22 ± 1.16	5.10 ± 1.07	6.43 ± 1.43	6.95 ± 1.83 [g]
CHO	9.51 ± 2.81	9.45 ± 1.83	5.82 ± 0.77	5.20 ± 0.88	5.12 ± 1.19	6.48 ± 1.36 [g]
PLA	8.15 ± 1.65	9.12 ± 1.95	7.13 ± 1.46	5.51 ± 1.22	6.62 ± 1.57	6.76 ± 1.22 [g]

Values are mean ± SE. [g]Significant change over time only, $P<0.05$.

Table 4.3. Plasma markers of muscle damage and inflammation

	CM	CHO	PLA
CHO oxidation ($g \cdot min^{-1}$)	0.159 ± 0.023	0.183 ± 0.028	0.079 ± 0.024 [b]
Fat oxidation ($g \cdot min^{-1}$)	0.093 ± 0.009	0.066 ± 0.010 [d]	0.102 ± 0.010
CHO EE ($kcals \cdot min^{-1}$)	0.669 ± 0.096	0.770 ± 0.119	0.332 ± 0.099 [b]
Fat EE ($kcals \cdot min^{-1}$)	0.843 ± 0.078	0.599 ± 0.093 [d]	0.926 ± 0.090

Average of 4 10-min gas collections (minutes 30, 90, 150 and 210) of recovery period. EE, energy expenditure. Values are mean ± SE. [b] PLA vs CM and CHO, $P<0.05$; [d] CHO vs CM and PLA, $P<0.05$

Table 4.4. Average substrate utilization during 4 h recovery period

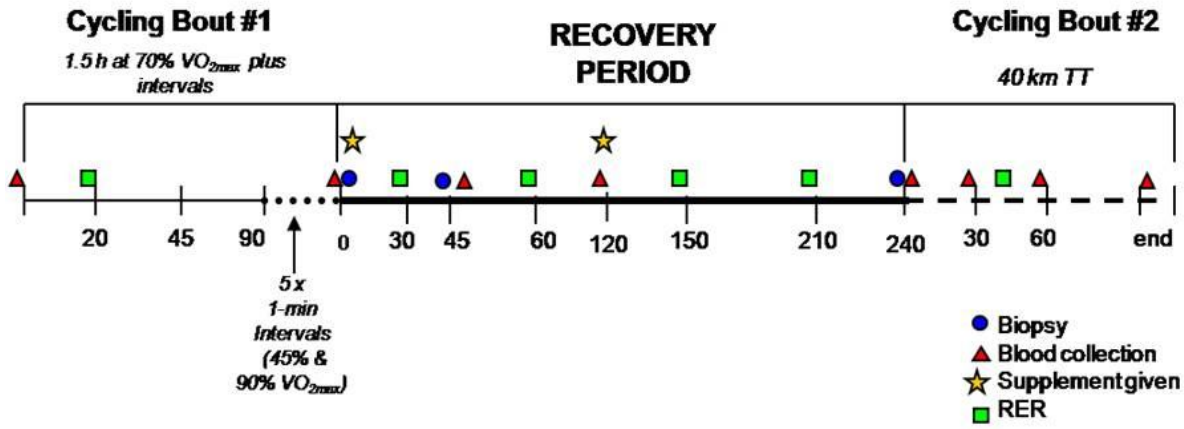

Figure 4.1. Experimental protocol

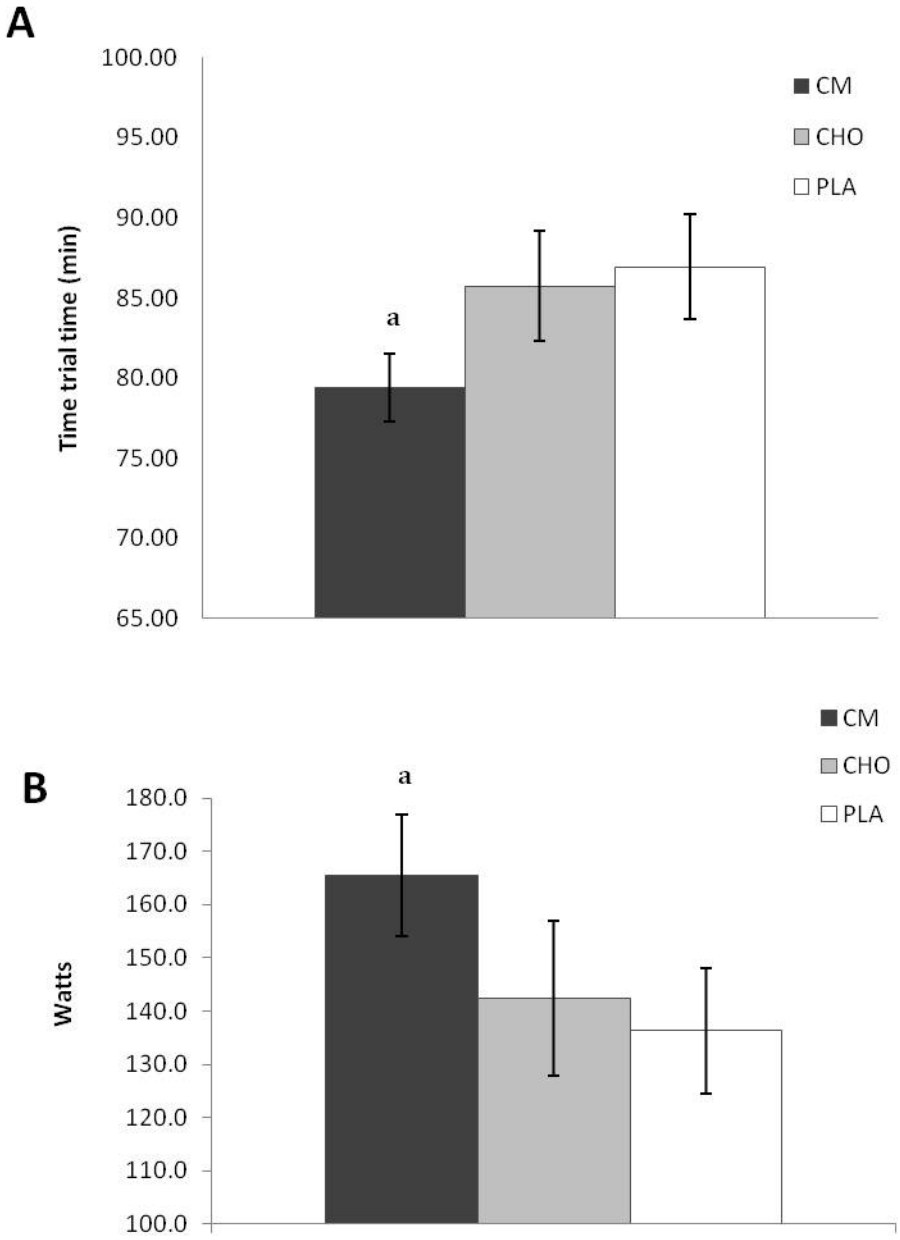

Figure 4.2. Time trial performance. A. Average time to complete a 40 km cycling time trial. **B.** Average Watts during time trial. Values are mean ± SE. Significant treatment differences: [a] CM vs CHO and PLA ($P<0.05$).

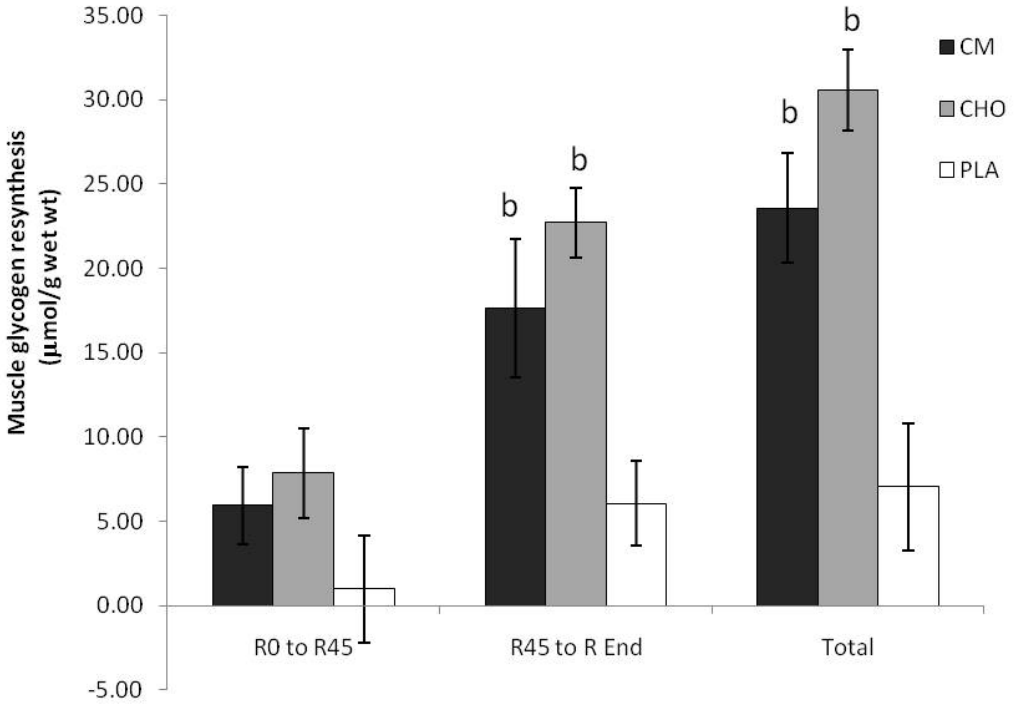

Figure 4.3. Muscle glycogen resynthesis during 4 h recovery. Biopsies were taken immediately (R0), 45 min (R45), and 4 h post-exercise (R End). Doses of supplement were provided immediately after the biopsy occurred at R0, and at 2 h into recovery. Resynthesis that occurred between R0 and R45 and R45 to R End are shown, as well as the total resynthesis over the 4 h period (R0 to R End). Values are mean ± SE. Significant treatment differences: [b] CM and CHO vs PLA ($P<0.05$).

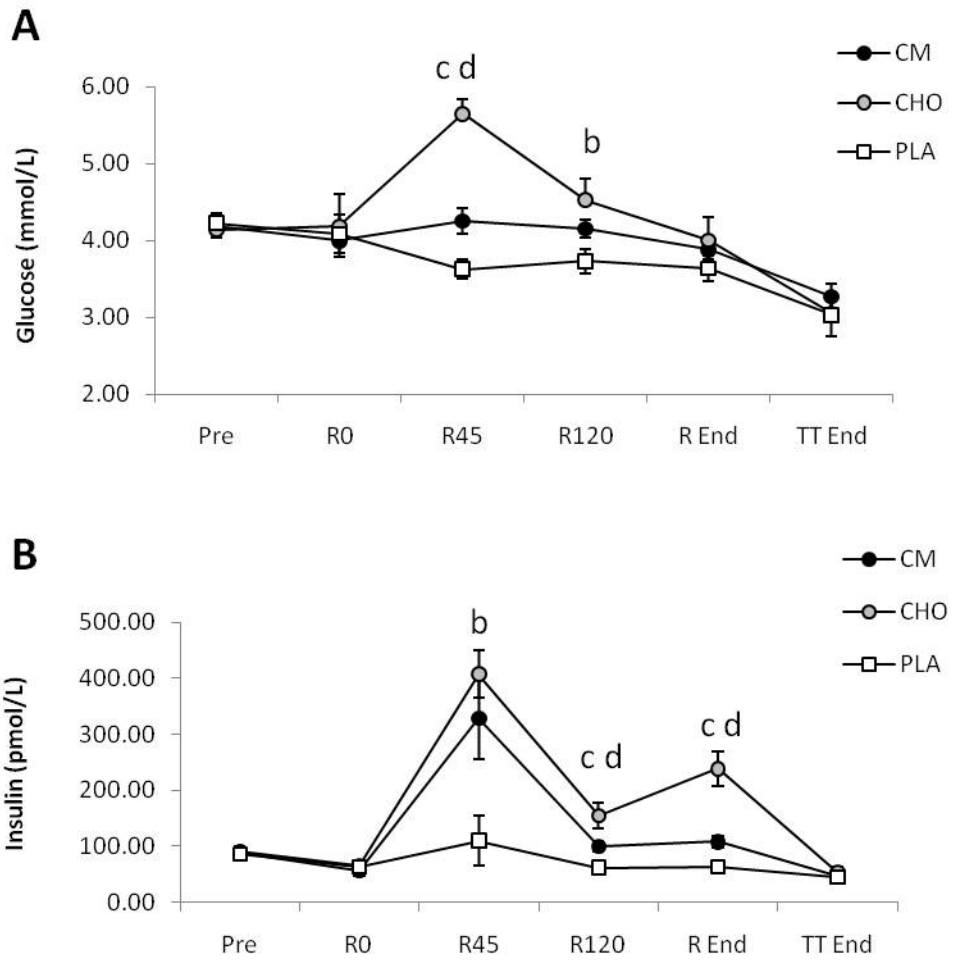

Figure 4.4. Plasma glucose and insulin. A. Plasma glucose measured at 6 time points throughout the exercise, recovery and time trial protocol. Significant treatment differences were found for CHO vs CM and PLA, and CM vs PLA ($P<0.05$). B. Plasma insulin measured at the same time points throughout the protocol. Significant treatment differences were found for CHO vs CM and PLA, and CM vs PLA ($P<0.05$). Values are mean ± SE. Significant treatment by time differences: [b] CM and CHO vs PLA; [c] CM vs PLA; [d] CHO vs CM and PLA ($P<0.05$).

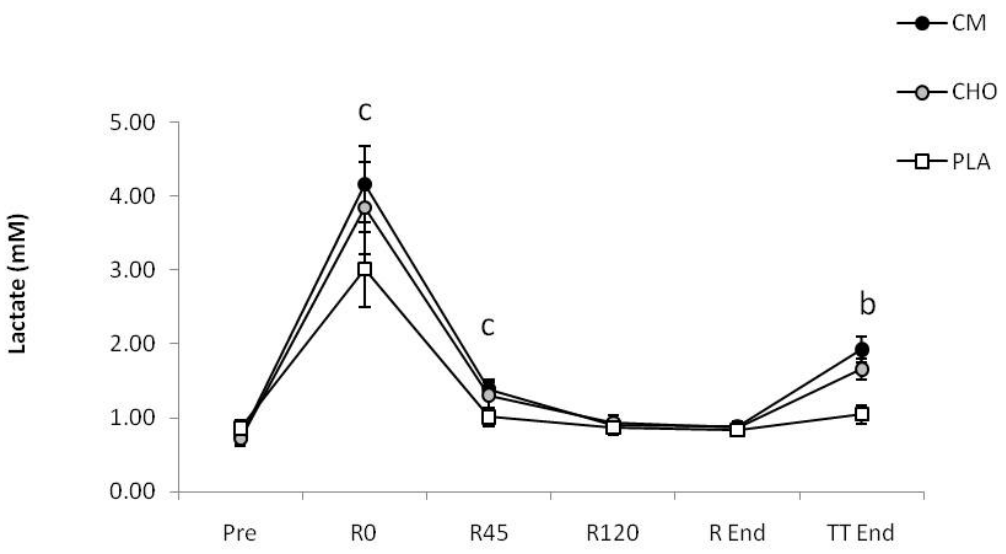

Figure 4.5. Blood lactate. Values are mean ± SE. Significant treatment differences were found for CM vs PLA (P<0.05). Significant treatment by time differences: [b] CM and CHO vs PLA; [c] CM vs PLA (P<0.05).

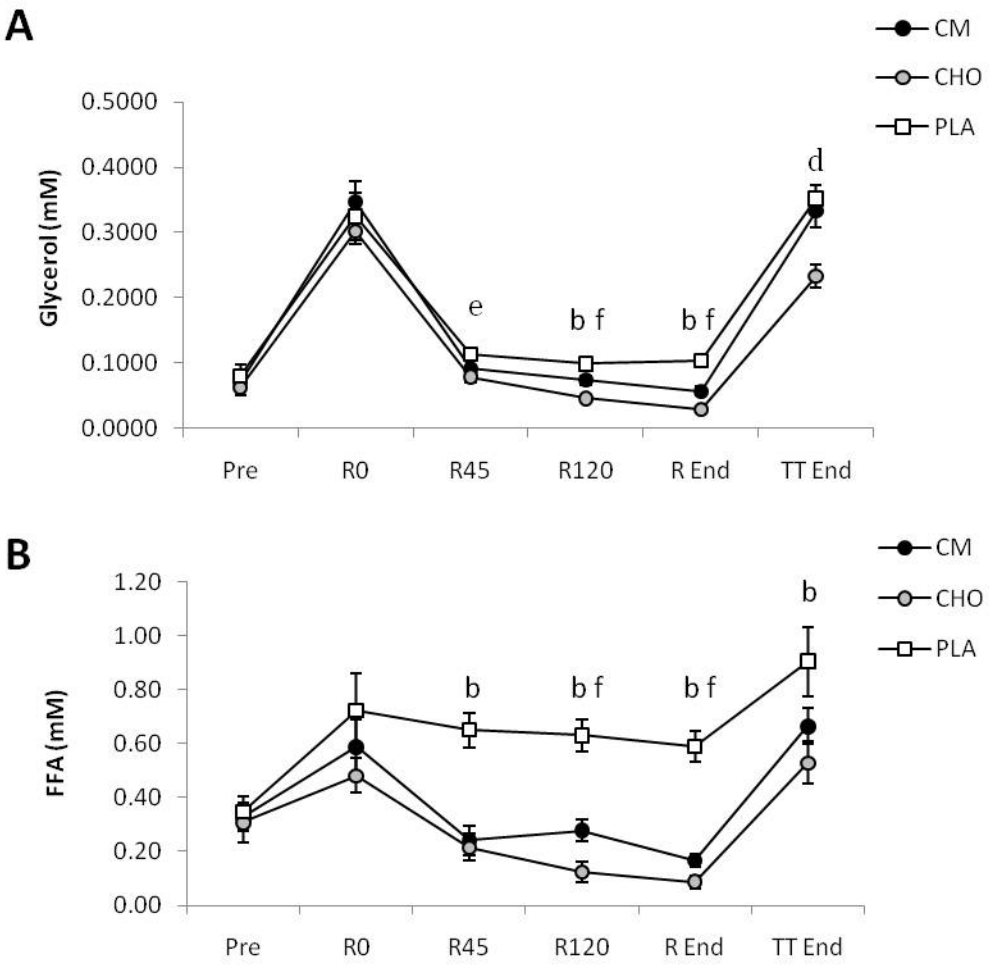

Figure 4.6. Plasma glycerol (A) and plasma free fatty acids (B). Values are mean ± SE. Significant treatment differences were found for PLA vs CM and CHO, and CM vs CHO ($P<0.05$). Significant treatment by time differences: [b] CM and CHO vs PLA; [d] CHO vs CM and PLA; [e] CHO vs PLA; [f] CM vs CHO ($P<0.05$).

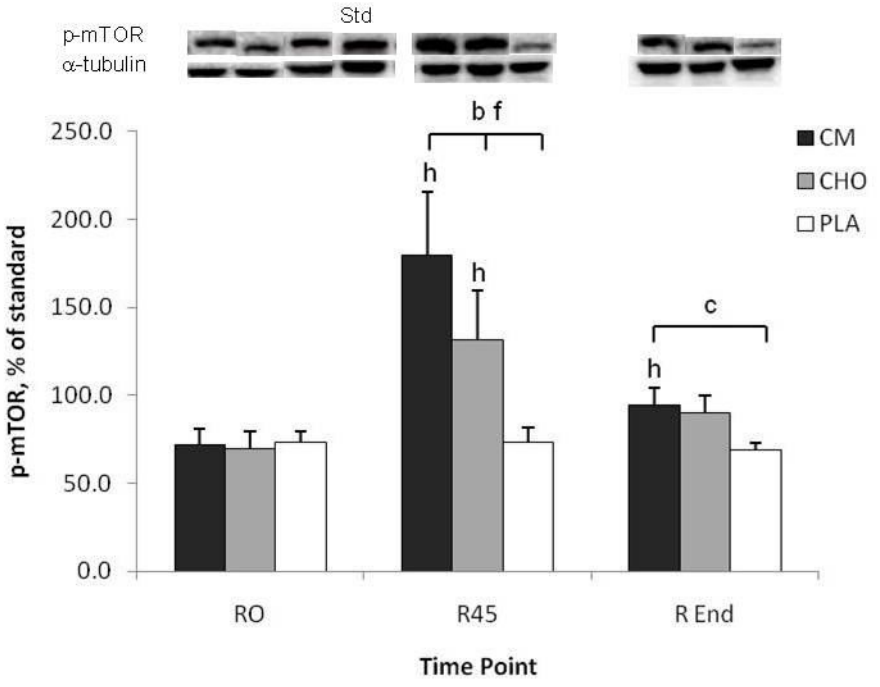

Figure 4.7. mTOR phosphorylation (Ser2448). A significant overall treatment difference existed between CM and PLA ($P<0.05$). Significant treatment by time differences: [b] CM vs CHO and PLA; [c] CM vs PLA; [f] CM vs CHO; [h] within-treatment significant difference from R0 ($P<0.05$).

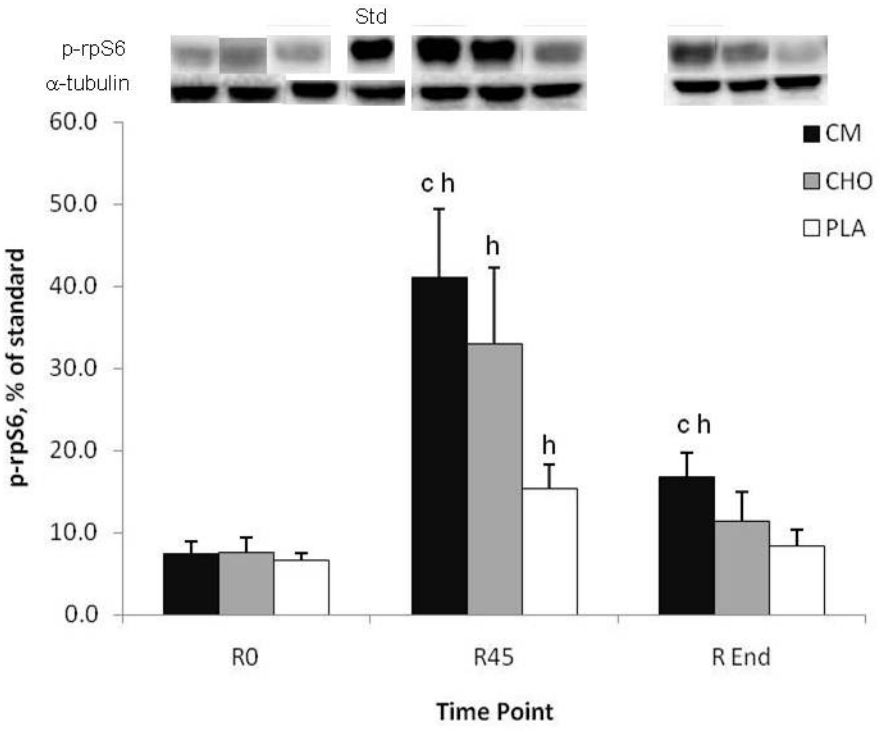

Figure 4.8. rpS6 (Ser 235/236) phosphorylation. A significant overall treatment difference existed between CM vs CHO and PLA ($P<0.05$). Significant treatment by time differences: [c] CM vs PLA; [h] within-treatment significant difference from R0 ($P<0.05$).

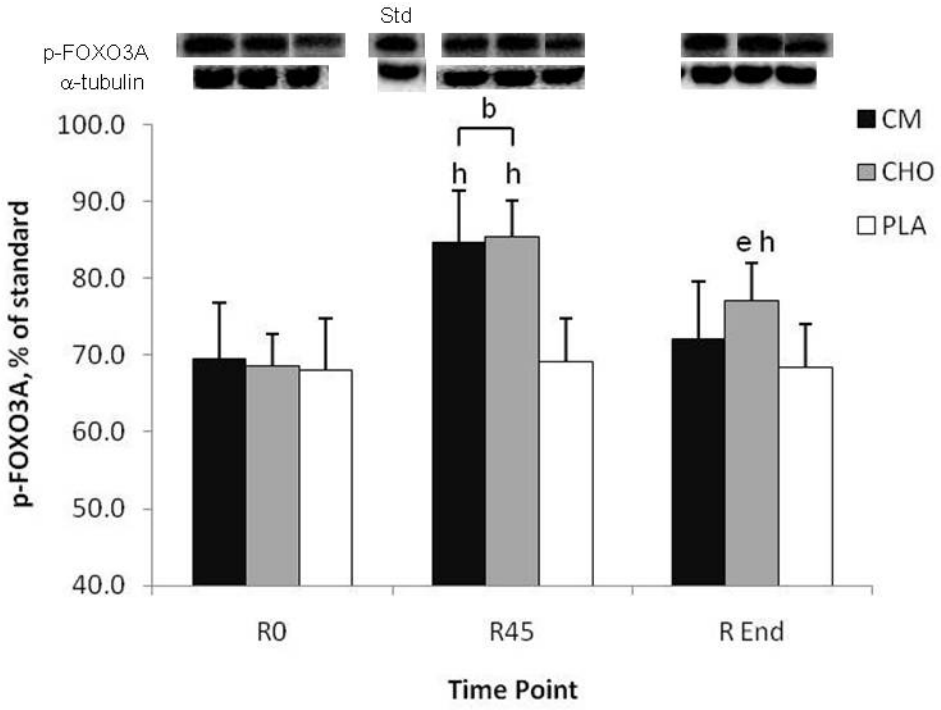

Figure 4.9. FOXO3A (Ser318/321) phosphorylation. A significant overall treatment difference existed between CHO and PLA ($P<0.05$). Significant treatment by time differences: [b] CM and CHO vs PLA; [e] CHO vs PLA; [h] within-treatment significant difference from R0 ($P<0.05$).

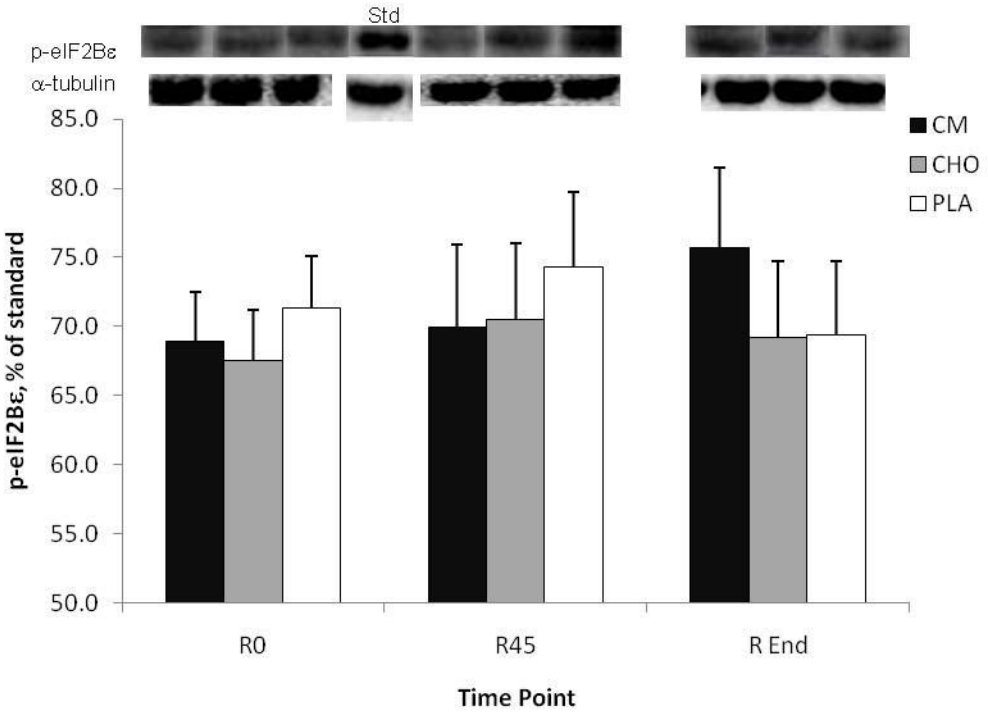

Figure 4.10. eIF2Bε (Ser539) phosphorylation. No significant treatment, time, or treatment x time differences were found.

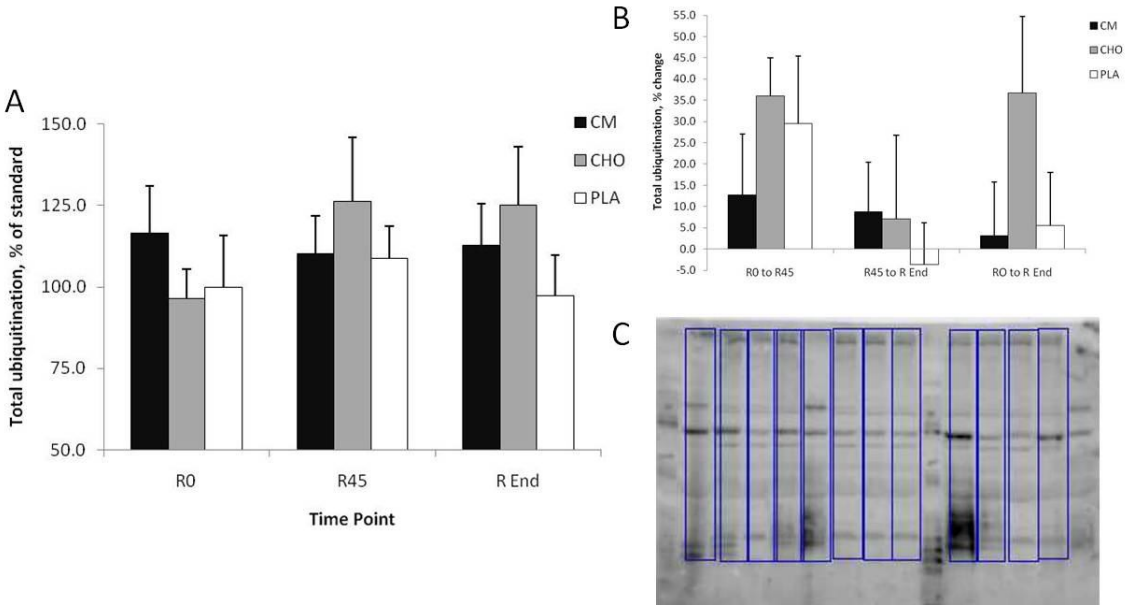

Figure 4.11. Ubiquitination. No significant treatment, time, or treatment x time differences were found. **A.** Total ubiquitination of tissue samples during the recovery period. **B.** Percent change in total ubiquitination. **C.** Example of Western blot measurement and quantification of ubiquitination.

Chapter V: Aerobic Exercise Training Adaptations are Increased by Post-Exercise Carbohydrate-Protein Supplementation

ABSTRACT

Carbohydrate-protein supplementation post-endurance exercise has been proposed to improve aspects of acute exercise recovery beyond that of a carbohydrate supplement alone. Provided as a supplement post-resistance exercise, it has been found to increase the rate of training adaptation. The purpose of the present study was to compare the effects of a carbohydrate and protein supplement in the form of chocolate milk (CM), an isocaloric carbohydrate supplement (CHO), and placebo (PLA) on cardiovascular and intramuscular adaptations that occurred in response to a 4.5 week aerobic exercise training program in healthy, untrained subjects. Thirty-two untrained males and females cycled for 1 h/, 5 d/wk for 4.5 wks at 75-80% of maximal oxygen consumption (VO_2max). Supplements were ingested immediately and 1 h after each session. VO_2max and lactate threshold were assessed at baseline, the midpoint of training, and end of the training period. Muscle biopsies were performed at baseline, midpoint, and end of the training period to assess changes in citrate synthase and succinate dehydrogenase activity, as well as for total PGC-1α content. Improvements in VO_2max were significantly greater in CM than CHO and PLA. Lactate threshold improved in all groups, with no differences found between treatments. Activity of citrate synthase and succinate dehydrogenase and total PGC-1α content increased in all groups over time with no significant differences between treatments. We conclude that improvements in VO_2max occur faster when supplementing with CM as compared with CHO or placebo, and that this faster rate of adaptation is systemic rather than cellular in nature.

INTRODUCTION

It is well established that aerobic endurance exercise training leads to cardiovascular, skeletal muscle and metabolic adaptations. Cardiovascular adaptations include increased stroke volume and cardiac output, which contributes greatly to increased maximal oxygen consumption (VO_2max) (9, 31). Skeletal muscle adaptations include increased mitochondrial content and activity of oxidative enzymes such as citrate synthase and succinate dehydrogenase (4, 8, 10, 13-16, 30). While many investigations have addressed the effects of endurance exercise training on such adaptations, few have examined the role of post-exercise nutritional supplementation in facilitating the adaptive process.

The beneficial effects of post-exercise supplementation in the form of carbohydrate (CHO) or carbohydrate-protein (CHO+PRO) supplements following an acute exercise bout has been the focus of many investigations. Several studies performed by our laboratory and others have demonstrated a greater improvement in acute exercise recovery with CHO+PRO supplementation compared to CHO alone (3, 24, 32, 38). Okazaki and colleagues (26) recently compared the effects of a CHO+PRO supplement to a placebo supplement in a model of chronic exercise training older male subjects who cycled for 60 min/d, 3 d/wk for 8 wk at 60-75% VO_2peak. They reported a two-fold increase in VO_2max in the CHO+PRO group compared to the placebo group (26). Thus, nutritional supplementation may increase the magnitude of training adaptations compared to the exercise stimulus alone. However, it was not possible to determine from their results if the increase in VO_2max was due to cellular or systemic adaptations. Moreover, their experimental design did not allow for macronutrient specific comparisons, as they did not include a CHO-only supplement.

Recently, chocolate milk (CM) has been investigated as a practical and effective carbohydrate and protein-containing supplement when ingested after an acute bout of strenuous endurance exercise. (5, 19, 29, 37). Therefore, the purpose of the present study was to investigate training adaptations that occurred after a 4.5 wk aerobic endurance

exercise (cycling) training program when supplementing after each daily exercise session with a CHO+PRO supplement in the form of CM, CHO or placebo. We aimed to determine if nutritional supplementation resulted in a greater increase in VO_2max and skeletal muscle oxidative enzyme activity. Although the exercise training was expected to induce positive adaptations in VO_2max and muscle oxidative capacity, we hypothesized that post-exercise CM supplementation would induce these adaptations faster than would occur with CHO or PLA supplementation.

METHODS

Subjects. Thirty-two healthy, recreationally active but untrained males and females (16 males and 16 females) between 18 and 35 years old completed the study. Subject characteristics are listed in Table 5.1. In order to be classified as recreationally active but not endurance trained, subjects could not have exercised regularly more than 3 h/wk over the last 2 years, and had VO_2max values of <40 mL/kg/min for females and <45 mL/kg/min for males. Potential subjects who did not meet these criteria were screened out of the study. A total of 36 subjects were admitted to the study, and 4 subjects voluntarily withdrew due to illness or work scheduling conflicts. Ten to twelve subjects per group were matched for age, gender and body composition, and then the subjects were randomized into one of the 3 treatment groups for the duration of the training period. Written informed consent was obtained from all subjects, and the study was approved by The University of Texas at Austin Institutional Review Board.

Research design and experimental protocol. This study followed a randomized, double-blinded, placebo controlled design. The protocol for the training period is shown in Table 5.2. The entire protocol period was 7 wk long and consisted of the following: a baseline testing week; the first and second weeks of training; a midpoint testing week in which subjects repeated the testing performed at baseline and trained 2 days; training weeks 3 and 4; and a partial week during which the end testing was performed. Subjects reported to the laboratory before the start of their training period on two occasions, once

for a baseline biopsy (described below), and again the following day for determination of lactate threshold (LT), maximal oxygen consumption (VO_2max) and maximal workload (W_{max}). This same test battery and schedule was repeated at the midpoint and at the end of the training period (Table 5.2).

The LT test was performed first, followed by the VO_2max test after a 5-min cool-down between the two tests. These tests were performed on a VeloTron DynaFit Pro cycle ergometer (RacerMate, Seattle, WA). LT was determined using 5-min stages beginning at 70 Watts (W) for males and 50 W for females. The Watts were increased by 25 W (males) or 20 W (females) each stage for the first 3-4 stages, followed by increases of 15 W (males) or 10 W (females) for the last 2-3 stages. A drop of blood was collected onto a lactate test strip after a finger stick during the last minute of each stage, and lactate levels were measured using a Lactate Pro LT-1710 lactate analyzer (Arkray, Inc., Minami-ku, Kyoto, Japan). LT was defined as the breakpoint at which lactate levels begin to rise above baseline levels. After the 5-min cool-down in which the subjects pedaled easily and drank water *ad libitum*, the VO_2max test began. VO_2max was measured using a TrueOne2400 system (ParvoMedics, Sandy, UT). Subjects breathed through a Hans Rudolph valve, with expired gases directed to a mixing chamber for analysis of oxygen (O_2) and carbon dioxide (CO_2). Outputs were directed to a computer for calculation of ventilation, O_2 consumption (VO_2), CO_2 production (VCO_2), and respiratory exchange ratio (RER) every 15 s.

The protocol for establishing VO_2max consisted of 2 min stages beginning at 125 W for males or 75 W for females. The work load was increased by 50 W (males) or 30 W (females) every 2 min until 275 W and 200 W, respectively. After this point, the workload increased 25 W (males) or 20 W (females) every minute until the subject could not continue to pedal despite constant verbal encouragement. The criteria used to establish VO_2max was a plateau in VO_2 with increasing exercise intensity and RER > 1.10.

Maximum power output in Watts was calculated from the VO_2max test data using the formula, adapted from Astrand and Rodahl (1):

$$W_{max} = (VO_2max\ mL - 300\ mL\ O_2) / 12.5\ W/mL\ O_2$$

The workload for the desired intensity level of the training rides (75% of VO_2max for the first 3.5 weeks and 80% for week 4) was then set as percentages of the W_{max} as follows:

$$W = [(VO_2max\ mL \times \%VO_2max\ desired) - 300\ mL\ O_2] / 12.5\ W/mL\ O_2$$

With the exception of determining W_{max} for the purposes of setting ride intensity levels, the baseline, midpoint and end testing consisted of the same tests in the same order.

During the training weeks, subjects reported to the training laboratory each morning after fasting overnight. All subjects began the rides as a group at the same time each day (6:00 AM or 7:30 AM), Monday – Friday. On Fridays, the subjects performed a cycling time trial (TT) that was designed to take about 1 h to complete. After each session including the TTs, subjects were provided one dose of supplement immediately post-exercise and were required to drink it in the laboratory. Subjects were then provided a second dose in an opaque to-go cup with a lid and straw and instructed to drink it 1 h later. They were also instructed to not ingest anything other than water until 1 h after ingesting the second dose.

The daily training rides and time trials were performed on Kona Dew bicycles (Kona, Ferndale, WA) mounted on CompuTrainer stationary trainers (RacerMate, Seattle, WA) interfaced with MultiRider III software (RacerMate, Seattle, WA). Six bicycles and CompuTrainers were interfaced with the system to allow for training groups of 6 subjects at one time. The bikes were set up based on each subject's physical measurements. The

CompuTrainers were calibrated each morning. To minimize thermal stress, air was circulated over the subjects with standing floor fans, and water was provided ad libitum. Investigators encouraged the subjects to drink as needed.

The first week of training served to get the subjects accustomed to cycling for prolonged periods. The first ride was 30 min in duration, the second was 40 min, the third ride, 50 min, and the fourth, 60 min. The first TT was a shortened version (16 km) of the 24-km TT course that was performed the next 3 wk and served to familiarize them with the concept of time trialing, while providing a shorter, more intense session. With the exception of 3 rides the first week, all rides on Monday – Thursday were 1 h in duration throughout the training period. The Friday TTs were ~50-70 min in duration.

Each training ride and TT began with a 10-min warm up at 60% VO_2max, after which the work rate was increased to elicit ~75% VO_2max for duration of each training ride. At the midpoint, VO_2max was reassessed and the workloads were adjusted accordingly to keep the subjects exercising at 75% VO_2max for the third week. For the fourth week, the intensity was increased to 80% VO_2max. A 5-min VO_2 measurement was performed at the beginning of each week to verify that the workload corresponded to the calculated intensity (%VO_2max) for each subject. The Wattage calculated for each subject was set by the investigators, and subjects were asked to maintain a cadence of ~70 rpms in order to maintain the Wattage. Subjects were not allowed to shift gears or vary their cadence during the rides on Monday – Thursday. For the TTs, subjects were allowed to shift gears on the bicycles in order to cycle the predetermined course as fast as possible.

Experimental beverages. After each daily session, subjects ingested the experimental beverages (CM, CHO, or PLA) immediately and l h post-exercise. The CM (Kirkland Organic Low-Fat Chocolate Milk, Costco Inc.) and CHO beverages were isocaloric and contained the same amount of fat. The placebo was an artificially flavored and artificially sweetened supplement that resembled the CHO beverage in taste and appearance, but

contained no calories. The energy and macronutrient composition of the beverages is shown in Table 5.3.

The amounts of supplement provided were stratified according to body weight ranges. Subjects weighing less than 63.6 kg (140 lbs) received 250 mL per supplement (197.5 kcals each), totaling 500 mL and 395 kcals. Subjects weighing between 63.6 kg (140 lbs) and 77.2 kg (170 lbs) received 300 mL per supplement (237 kcals), totaling 600 mL and 474 kcals. Subjects weighing between 77.2 kg (170 lbs) and 90.9 kg (200 lbs) received 350 mL per supplement (277 kcals), totaling 700 mL and 554 kcals. Subjects weighing over 90.9 kg (200 lbs) received 375 mL per supplement (296.5 kcals), totaling 750 mL and 593 kcals. For the CHO treatment, the amount of carbohydrate (dextrose) and fat (canola oil) matched that provided in the CM as measured for the individual's weight range. Both the CHO and Placebo beverages were flavored with grape-flavored non-caloric Kool-Aid. The CM supplement provided an average of 0.94 g carbohydrate, 0.31 g protein, and 0.17 g fat per kg body weight. The CHO supplement provided an average of 1.25 g carbohydrate and 0.17 g fat per kg body weight.

Diet and exercise. Subjects were asked to keep their diets and activity levels consistent for the duration of the study (i.e.; no significant changes in caloric intake, dietary habits, or activity levels outside of the study's training sessions). The subjects were instructed to maintain a dietary and activity log for the 2 days prior to their baseline, midpoint and end biopsies and testing. They were also instructed to keep a 2-day dietary and activity log each week on Wednesdays and Thursdays before the Friday TT so that compliance in keeping their normal dietary habits and activity levels consistent prior to the TTs could be verified. In addition, the subjects were asked to replicate their diet and activity on the days the logs are kept, such that the diet and activity was the same on the 2 days prior to each TT or each biopsy session. Subjects were required to turn the logs in each week, and the logs were analyzed for macronutrient composition and total caloric intake using Nutritionist V Dietary Analysis Software (First Data Bank, Inc, San Bruno, CA). All

subjects complied with the diet and activity requirements. Subjects were instructed to arrive at the laboratory having fasted overnight for 12 h for every exercise session and laboratory visit, except for the LT and VO_2max testing sessions.

Lactate threshold and VO_2max. These measures were determined at baseline, the midpoint and at the end of the 4.5 wk training period, as shown in Table 5.2. The protocol for these tests is detailed above.

Time trial performance. Each Friday of training weeks 2, 3, and 4, subjects performed a 25-km time trial. The 25-km distance was selected because it was a reasonable distance to cover in 1 h or less when riding at a high intensity. Subjects were asked to ride the course as fast and intensely as they felt was possible, as if it were a race. During all TTs, subjects were blinded to their elapsed time or distance covered.

Muscle biopsy procedure. Muscle biopsies were taken at baseline, the midpoint, and the end of the training period, as shown in Table 5.2. Prior to each biopsy, the subject's thigh was cleansed with 10% betadine solution and 1.4 mL of 1% Lidocaine Hydrochloride (Elkins-Sinn, Inc., Cherry Hill, NJ) was injected to prepare the leg for the muscle biopsy. Approximately ~45-60 mg wet wt of tissue was taken from the vastus lateralis through a 5-8 mm incision made through the skin and fascia, 6 inches from the midline of the thigh on the lateral side and 2.5 inches above the patella. The tissue samples were trimmed of adipose and connective tissue and immediately frozen in liquid nitrogen at -80^{o}C for subsequent analysis.

Muscle tissue processing. The muscle samples were weighed and cut in half. One half of the tissue sample was used for determination of citrate synthase and succinate dehydrogenase activity, and the other half for measurement of total PGC-1α content. For the enzymatic analyses, samples were homogenized in ice-cold buffer containing 20 mM Hepes, 2 mM EGTA, 50 mM sodium fluoride, 100 mM potassium chloride, 0.2 mM

EDTA, 50 mM glycerophosphate, 1 mM DTT, 0.1 mM PMST, 1 mM benzamidine, and 0.5 mM sodium vanadate (pH 7.4) at a dilution of 1:10. Homogenization was performed on ice using 3 x 5 s bursts with a Caframo RZRl Stirrer (Caframo Limited, Warton, Ontario, Canada). The homogenate was immediately centrifuged at 14,000 g for 10 min at 4°C, the supernatant aliquoted to storage tubes for each assay and stored at -80°C. For determination of total PGC-1α content, the tissue samples were homogenized at a dilution of 1:10 in a modified RIPA buffer based on a previously described protocol (39) containing: 50 mM Tris-HCL (pH 7.4); 150 mM NaCl (pH 7.4); 1% each Igepal CA-630 and sodium deoxycholate; 1 mM each EDTA (pH 7.4), Na_3VO_4 (pH 10), NaF, and phenylmethylsulfonyl fluoride; 1 μg/ml each aprotinin, leupeptin and pepstatin. Homogenization was performed on ice using 4 x 5 s bursts with a Caframo RZRl Stirrer (Caframo Limited, Warton, Ontario, Canada). The homogenates were sonicated on ice for 10 s and then centrifuged at 5,000 g for 20 min at 4°C. The supernatant was aliquoted to storage tubes and stored at -80°C. Protein concentration was determined from the supernatant using a modified version of the Lowry Assay (21) for each sample, and was measured before each of the assays were performed.

PGC-1α content. Total PGC-1α content was determined by Western blotting. Total α–tubulin content was also determined as a housekeeping protein. Aliquots of homogenized muscle sample supernatants and standards were slowly thawed over ice and diluted 1:1 with sample buffer containing 1.25M Tris, pH 6.8, glycerol, 20% SDS, 2-mercaptoethanol, 0.25% bromophenol blue solution, and deionized water. Samples containing 70 μg of total protein were separated on 10% polyacrylamide gels by SDS-PAGE for 75 min at 200 V (Bio-Rad Laboratories, Hercules, CA.) After electrophoresis, the gels were electrotransferred using a semi-dry transfer cell (Bio-Rad Laboratories, Hercules, CA.) using 25 V for 18 min to 0.4 μm polyvinylidine fluoride (PVDF) membranes (Millipore, Bedford, MA). The membranes were blocked in TTBS (TBS, 50 mM Tris, 150 mM NaCl, containing 0.1% Tween-20), and 10% nonfat dry milk for 2 h at room temperature on a rocking platform at medium speed. The membranes were then

washed in 1x TTBS 3 times for 5 min each wash. Using the molecular weight markers visible on the membranes as a guide, the membranes were cut at the 75 kD marker. The upper section was used for detection of PGC-1α, and the lower section was used to detect α-tubulin. Each membrane section was incubated overnight at 4°C on a rocking platform at low speed with antibodies directed against PGC-1α (no. 515667, EMD Calbiotech/Merck KGaA, Darmstadt, Germany), and α-tubulin (no. 2144, Cell Signaling, Danvers, MA). The antibodies were diluted 1:1000 for PGC-1α, and 1:900 for α-tubulin in TTBS containing 2% nonfat dry milk. Following the overnight incubation, membranes were washed three times with TTBS for 5 min each wash and incubated for 1.5 h with a secondary antibody (goat anti-rabbit, HRP-linked IgG, no. 7074, Cell Signaling, Danvers, MA). Dilutions were 1:7500 for PGC-1α and 1:1000 for α-tubulin. The immunoblots were visualized by enhanced chemiluminescence (Perkin Elmer, Boston, MA) using a Bio-Rad ChemiDoc detection system, and the mean density of each band was quantified using Quantity One 1-D Analysis software (Bio-Rad Laboratories, Hercules, CA.). A molecular weight ladder (Precision Plus Protein Standard, Bio-Rad) and a rodent internal control standard prepared from insulin-stimulated mixed skeletal muscle were also included on each gel. All blots were compared with the rodent control standard and the values of each sample were represented as a percent of standard for each blot.

Citrate synthase and succinate dehydrogenase. Citrate synthase (CS) activity was determined according to the protocol of Srere et al. (35) on the homogenates after further dilution of 1:10 (wt/vol) with 0.1 M Tris-HCI and 0.4% Triton X-100 buffer (pH 8.1). The rate of appearance of DTNB was determined spectrophotometrically over 5 min at 412 nm and 37^{o} C using a Beckman DU 640 spectrophotometer (Fullerton, CA), and was proportional to CS activity. Succinate dehydrogenase (SDH) activity was measured according to the method of Lowry and Passonneau (20). The amount of NADH produced during a 5 min incubation time was read on a Varian Cary Eclipse fluorometer with an excitation wavelength of 340 nm and emission wavelength of 450 nm (Varian, Inc., Palo

Alto, CA) and corresponded to SDH activity in the sample. CS and SDH activities were expressed as μmols/g/min protein.

Statistical analyses. VO_2max, LT, TT time and TT Watts were analyzed using two-way (treatment x time) analysis of variance (ANOVA) for repeated measures. Muscle enzyme activity and PGC-1α content was analyzed using two-way (treatment x time) analysis of variance (ANOVA) for repeated measures. For all measures, post hoc analysis was performed when significance was found using Least Significant Difference (LSD). Differences were considered significant at $P<0.05$. Data were expressed as mean ± SE. All statistical analyses were performed using SPSS version 16.0 statistical software (SPSS Inc., Chicago, IL).

RESULTS

VO_2max. Absolute and relative changes in VO_2max are shown in Figure 5.1. No significant differences existed between the groups at baseline. All treatment groups experienced significant increases in absolute and relative VO_2max over the 4.5 wk training period. The change in both absolute and relative VO_2max was significantly greater in the CM group compared to CHO and PLA. The increases in the CHO and PLA groups were not statistically different from each other (Figure 5.1).

Lactate threshold and time trial performance. Although lactate threshold increased significantly over time in all 3 treatment groups, no significant treatments differences were observed (Table 5.4). Decreases in weekly TT time, and increases in average TT Watts, were significant in all groups over the duration of the training period, but no significant treatment differences were detected (Table 5.5).

Citrate synthase and succinate dehydrogenase. No significant treatment or treatment by time effects were found for CS or SDH (Figure 5.2A and B). Significant time effects existed for both enzymes in all treatment groups ($P<0.05$).

PGC-1α. Changes in total PGC-1α content are shown in Figure 5.3. No significant treatment or treatment by time effects were found. However, significant time effects existed in all treatment groups ($P<0.05$).

DISCUSSION

The key finding of the present study was that the increase in VO_2max was significantly greater in the CM group than the CHO or PLA groups. The average increase in absolute VO_2max for the CM group was 12.5% higher than baseline levels, a two-fold improvement over the increase found in the CHO and PLA groups. The average absolute VO_2max (L/min) increase for all subjects and treatment groups combined was 9.2% over the 4.5 wk training period, which is in agreement with other investigations of aerobic training and VO_2max improvements using a similar time period (27, 34).

It has been established that the primary determinants of VO_2max are an increased ability of the cardiovascular system to transport oxygen to the working skeletal muscle, and the improved ability of the muscle to utilize the delivered oxygen. The former is a result of increased stroke volume, which improves cardiac output; the latter is determined by the increases in oxidative enzymes and mitochondrial content (9, 31). We measured the activity of two key oxidative enzymes that are indicative of muscle oxidative capacity, CS and SDH. Both are found in the mitochondria and are key enzymes of the Kreb's cycle, and each has been demonstrated to increase in response to endurance training. (4, 8, 10, 13, 14, 16, 22, 30). We also measured total content of the transcription coactivator PGC-1α (peroxisome proliferator-activated receptor γ coactivator-1α) as a marker for increased mitochondrial biogenesis. PGC-1α is a transcriptional coactivator of transcription factor PPARγ and together, they regulate the expression of genes that encode mitochondrial proteins. An acute bout of exercise or stimulated skeletal muscle contraction induces an increase in both PGC-1α mRNA and protein in skeletal muscle (2,

12, 17, 36), and it has been shown that increased PGC-1α activation and total protein amount leads to increased mitochondrial biogenesis (39).

In the present study, we demonstrated that the activity of CS and SDH and the total content of PGC-1α increased significantly in response to 4.5 wks of training. However, no significant treatment differences in those measures were detected. There was a slight but non-significant trend for a greater increase in CS and SDH activity in CM compared to CHO and PLA. It may be that the training period was not long enough to detect any potential differences that could emerge in response to chronic nutritional supplementation. Thus, our results suggest that the greater VO_2max improvements with CM supplementation are most likely due to cardiovascular adaptations rather than increases in oxidative enzymes or in mitochondrial biogenesis.

As mentioned previously, endurance training leads to an adaptive increase in cardiac output, and this increase is due to augmented stroke volume (9). While we did not measure these variables in the present study, our results suggest that the significant improvement in VO_2max in the CM group is likely due to increased stroke volume and cardiac output, which is likely due to increased plasma volume. Plasma volume expansion is a hallmark of aerobic endurance training (33), and is directly associated with increased plasma albumin content. Increased albumin in the plasma causes water to be retained in the vasculature due to increases in the colloid osmotic pressure gradient (6, 11). Hepatic albumin synthesis has been shown to increase in response to endurance exercise training (23, 40). It has been previously demonstrated that plasma albumin content was increased 23 h after an acute bout of cycling exercise when CHO+PRO supplementation was provided post-exercise compared to placebo (25). These results, along with the findings of the present study, suggest that hepatic albumin synthesis may have been increased to a greater extent in the CM group compared to the CHO or PLA groups, and contributed to the significantly greater increase in VO_2max in the CM group.

Okazaki and colleagues (26) recently demonstrated that CHO+PRO supplementation provided immediately after daily cycling exercise training in older male subjects increased stroke volume and plasma volume compared to a placebo group. Their subjects cycled for 60 min/d, 3 d/wk for 8 wk at 60-75% VO_2peak and ingested either CHO+PRO or placebo immediately post-exercise each session. VO_2peak increased 3.3% in the control group and 6.8% in the CHO+PRO group, with significant stroke volume and plasma volume increases only found in the CHO+PRO group (26). In the present study, we extend the findings of Okazaki and colleagues (26) by demonstrating that the effect of nutritional supplementation on VO_2max increases is nutrient specific. In comparing CM against an isocaloric CHO only supplement and a placebo, we have shown that the increased VO_2max response is not due to simply providing calories post-exercise. In the present study, the VO_2max increase in the CHO and PLA groups was not significantly different. Thus, these results suggest that the benefit from a CHO+PRO or CM supplement in improving VO_2max is due to the combined ingestion of carbohydrate and protein.

In addition to well-documented increases in VO_2max with training, it is known that lactate threshold improves with endurance exercise training of moderate to high intensity (28). In the current study, LT improved significantly over the 4.5 wks of training, although there were no significant treatment differences detected (Table 5.4). It has been shown that the respiratory capacity of the muscle is the key determinant of LT (18). Given that muscle oxidative enzyme activity and PGC-1α content increased significantly over time without demonstrating a treatment effect, it would be expected that LT would follow a parallel pattern. Therefore, the results suggest that while LT is increased by exercise training in parallel with muscle oxidative capacity, it likewise may not be affected by nutritional supplementation.

In order to assess exercise performance as a measure of adaptive progress in the groups, subjects performed 25 km time trials on Fridays of weeks 2, 3 and 4. We hypothesized

that the CM group would adapt to the training stimulus faster than the CHO or PLA groups and would thus be able to cycle at a faster, "race" pace during these time trials than the CHO or PLA groups. Although all groups significantly improved their time to completion over the weeks of training, there were no treatment differences. Previous investigations have established that endurance performance is related to oxidative capacity of the muscle (14, 15), and that lactate threshold is a better determinant of endurance performance than VO_2max (7). Given that muscle oxidative capacity and lactate threshold increased in parallel in all treatment groups, it may have been difficult to detect treatment differences in time trial performance in the present study, especially in previously untrained, non-competitive subjects who were unaccustomed to time trialing at a race-like pace.

In summary, CM supplementation increased adaptations to a 4.5 wk aerobic exercise training program, demonstrated by greater improvements in VO_2max compared to CHO or PLA. This is likely due to increased plasma volume and thus, increased cardiac output. Muscle oxidative capacity, lactate threshold, and time trial performance improved significantly in all groups, with no differences found between treatments. Therefore, our findings suggest that improvements in VO_2max occur faster when supplementing with CM as compared with CHO or placebo, and that this faster rate of adaptation is systemic rather than cellular in nature. We conclude that CM is an effective post-exercise recovery supplement that can induce greater increases in aerobic training adaptations in healthy, untrained humans.

REFERENCES

1. Åstrand PO, and Rodahl K. Textbook of work physiology: Physiological bases of exercise. . New York: McGraw-Hill Book Company, 1977.
2. Baar K, Wende AR, Jones TE, Marison M, Nolte LA, Chen MAY, Kelly DP, and Holloszy JO. Adaptations of skeletal muscle to exercise: rapid increase in the transcriptional coactivator PGC-1. FASEB J 16: 1879-1886, 2002.
3. Berardi JM, Price TB, Noreen EE, and Lemon PW. Postexercise muscle glycogen recovery enhanced with a carbohydrate-protein supplement. Med Sci Sports Exerc 38: 1106-1113, 2006.
4. Carter SL, Rennie CD, Hamilton SJ, and Tarnopolsky MA. Changes in skeletal muscle in males and females following endurance training. Can J Physiol Pharmacol 79: 386-392, 2001.
5. Cockburn E, Hayes PR, French DN, Stevenson E, and St Clair Gibson A. Acute milk-based protein-CHO supplementation attenuates exercise-induced muscle damage. Appl Physiol Nutr Metab 33: 775-783, 2008.
6. Convertino VA, Brock PJ, Keil LC, Bernauer EM, and Greenleaf JE. Exercise training-induced hypervolemia: role of plasma albumin, renin, and vasopressin. J Appl Physiol 48: 665-669, 1980.
7. Coyle EF, Coggan AR, Hopper MK, and Walters TJ. Determinants of endurance in well-trained cyclists. J Appl Physiol 64: 2622-2630, 1988.
8. De Bock K, Derave W, Eijnde BO, Hesselink MKC, Koninckx E, Rose AJ, Schrauwen P, Bonen A, Richter EA, and Hespel PJ. Effect of training in the fasted state on metabolic responses during exercise with carbohydrate intake. J Appl Physiol 01195.02007, 2008.
9. Ekblom B, Astrand PO, Saltin B, Stenberg J, and Wallstrom B. Effect of training on circulatory response to exercise. J Appl Physiol 24: 518-528, 1968.
10. Fernstrom M, Tonkonogi M, and Sahlin K. Effects of acute and chronic endurance exercise on mitochondrial uncoupling in human skeletal muscle. J Physiol 554: 755-763, 2004.
11. Gillen CM, Lee R, Mack GW, Tomaselli CM, Nishiyasu T, and Nadel ER. Plasma volume expansion in humans after a single intense exercise protocol. J Appl Physiol 71: 1914-1920, 1991.
12. Goto M, Terada S, Kato M, Katoh M, Yokozeki T, Tabata I, and Shimokawa T. cDNA Cloning and mRNA analysis of PGC-1 in epitrochlearis muscle in swimming-exercised rats. Biochem Biophys Res Commun 274: 350-354, 2000.
13. Henriksson J, and Reitman JS. Time course of changes in human skeletal muscle succinate dehydrogenase and cytochrome oxidase activities and maximal oxygen uptake with physical activity and inactivity. Acta Physiol Scand 99: 91-97, 1977.
14. Holloszy JO. Biochemical Adaptations in Muscle: Effects of exercise on mitochondrial oxygen uptake and respiratory enzyme activity in skeletal muscle. J Biol Chem 242: 2278-2282, 1967.
15. Holloszy JO, and Coyle EF. Adaptations of skeletal muscle to endurance exercise and their metabolic consequences. J Appl Physiol 56: 831-838, 1984.

16. Holloszy JO, Oscai LB, Don IJ, and Mole PA. Mitochondrial citric acid cycle and related enzymes: adaptive response to exercise. Biochem Biophys Res Commun 40: 1368-1373, 1970.
17. Irrcher I, Adhihetty PJ, Sheehan T, Joseph A-M, and Hood DA. PPARgamma coactivator-1alpha expression during thyroid hormone- and contractile activity-induced mitochondrial adaptations. Am J Physiol Cell Physiol 284: C1669-1677, 2003.
18. Ivy JL, Withers RT, Van Handel PJ, Elger DH, and Costill DL. Muscle respiratory capacity and fiber type as determinants of the lactate threshold. J Appl Physiol 48: 523-527, 1980.
19. Karp JR, Johnston JD, Tecklenburg S, Mickleborough TD, Fly AD, and Stager JM. Chocolate Milk as a Post-Exercise Recovery Aid. International Journal of Sport Nutrition and Exercise Metabolism 16: 78-91, 2006.
20. Lowry H, and Passonneau JV. A Flexible System of Enzymatic Analysis. New York: Academic, 1972.
21. Lowry OH, Rosebrough NJ, Farr AL, and Randall RJ. Protein measurement with the folin phenol reagent. J Biol Chem 193: 265-275, 1951.
22. Morton JP, Croft L, Bartlett JD, MacLaren DPM, Reilly T, Evans L, McArdle A, and Drust B. Reduced carbohydrate availability does not modulate training-induced heat shock protein adaptations but does upregulate oxidative enzyme activity in human skeletal muscle. J Appl Physiol 106: 1513-1521, 2009.
23. Nagashima K, Cline GW, Mack GW, Shulman GI, and Nadel ER. Intense exercise stimulates albumin synthesis in the upright posture. J Appl Physiol 88: 41-46, 2000.
24. Niles ES, Lachowetz T, Garfi J, Sullivan W, Smith JC, Leyh BP, and Headley SA. Carbohydrate-Protein Drink Improves Time to Exhaustion after Recovery from Endurance Exercise. JEPonline 4: 45-52, 2001.
25. Okazaki K, Goto M, and Nose H. Protein and carbohydrate supplementation increases aerobic and thermoregulatory capacities. The Journal of Physiology 587: 5585-5590, 2009.
26. Okazaki K, Ichinose T, Mitono H, Chen M, Masuki S, Endoh H, Hayase H, Doi T, and Nose H. Impact of protein and carbohydrate supplementation on plasma volume expansion and thermoregulatory adaptation by aerobic training in older men. J Appl Physiol 107: 725-733, 2009.
27. Phillips SM, Green HJ, Tarnopolsky MA, Heigenhauser GJ, and Grant SM. Progressive effect of endurance training on metabolic adaptations in working skeletal muscle. Am J Physiol Endocrinol Metab 270: E265-272, 1996.
28. Poole DC, and Gaesser GA. Response of ventilatory and lactate thresholds to continuous and interval training. J Appl Physiol 58: 1115-1121, 1985.
29. Pritchett K, Bishop P, Pritchett R, Green M, and Katica C. Acute effects of chocolate milk and a commercial recovery beverage on postexercise recovery indices and endurance cycling performance. Appl Physiol Nutr Metab 34: 1017-1022, 2009.

30. Puntschart A, Claassen H, Jostarndt K, Hoppeler H, and Billeter R. mRNAs of enzymes involved in energy metabolism and mtDNA are increased in endurance-trained athletes. Am J Physiol Cell Physiol 269: C619-625, 1995.
31. Saltin B, Blomqvist G, JH Mitchell J, Johnson Jr R, Wildenthal K, and Chapman C. A Longitudinal Study of Adaptive Changes in Oxygen Transport and Body Composition. Circulation 38: VII 1-78, 1968.
32. Saunders M, Kane M, and Todd K. Effects of a Carbohydrate-Protein Beverage on Cycling Endurance and Muscle Damage. Med Sci Sports Exc 36: 1233-1238, 2004.
33. Sawka MN, Convertino VA, Eichner ER, Schnieder SM, and Young AJ. Blood volume: importance and adaptations to exercise training, environmental stresses, and trauma/sickness. Med Sci Sports Exerc 32: 332-348, 2000.
34. Spina RJ, Chi MM, Hopkins MG, Nemeth PM, Lowry OH, and Holloszy JO. Mitochondrial enzymes increase in muscle in response to 7-10 days of cycle exercise. J Appl Physiol 80: 2250-2254, 1996.
35. Srere PA. Citrate synthase. Methods Enzymol 13: 3-5, 1969.
36. Terada S, Goto M, Kato M, Kawanaka K, Shimokawa T, and Tabata I. Effects of low-intensity prolonged exercise on PGC-1 mRNA expression in rat epitrochlearis muscle. Biochem Biophys Res Commun 296: 350-354, 2002.
37. Thomas K, Morris P, and Stevenson E. Improved endurance capacity following chocolate milk consumption compared with 2 commercially available sport drinks. Appl Physiol Nutr Metab 34: 78-82, 2009.
38. Williams MB, Raven PB, Fogt DL, and Ivy JL. Effects of Recovery Beverages on Glycogen Restoration and Endurance Exercise Performance. Journal of Strength and Conditioning Research 17: 12-19, 2003.
39. Wright DC, Han D-H, Garcia-Roves PM, Geiger PC, Jones TE, and Holloszy JO. Exercise-induced Mitochondrial Biogenesis Begins before the Increase in Muscle PGC-1{alpha} Expression. J Biol Chem 282: 194-199, 2007.
40. Yang RC, Mack GW, Wolfe RR, and Nadel ER. Albumin synthesis after intense intermittent exercise in human subjects. J Appl Physiol 84: 584-592, 1998.

	All subjects (32)	CM (11)	CHO (11)	PLA (10)
Age (y)	22.0 ± 0.5	22.1 ± 0.7	21.3 ± 0.9	22.6 ± 1.0
Weight (kg)	71.7 ± 2.4	70.9 ± 5.1	71.2 ± 3.1	73.2 ± 4.5
Height (cm)	168.6 ± 1.5	169.1 ± 2.3	168.0 ± 2.7	168.8 ± 3.1
VO_2max ($L \cdot min^{-1}$)	2.6 ± 0.2	2.7 ± 0.3	2.6 ± 0.2	2.6 ± 0.2
VO_2max ($mL \cdot kg \cdot min^{-1}$)	35.9 ± 1.9	36.8 ± 1.4	35.7 ± 2.2	35.2 ± 2.1

Values are mean ± SE.

Table 5.1. Subject characteristics at baseline

	Mon	Tue	Wed	Thurs	Fri	Sat	Sun
Baseline	LT/VT and VO_2max testing; biopsy						
Week 1 (75% VO_2max)	30 min	40 min	50 min	60 min	TT	Rest	Rest
Week 2 (75% VO_2max)	60 min	60 min	60 min	60 min	TT	Rest	Rest
Midpoint	LT/VT and VO_2max testing; biopsy			60 min	60 min	Rest	Rest
Week 3 (75% VO_2max)	60 min	60 min	60 min	60 min	TT	Rest	Rest
Week 4 (80% VO_2max)	60 min	60 min	60 min	60 min	TT	Rest	Rest
End	LT/VT and VO_2max testing; biopsy						

Table 5.2. Protocol for training period

	CM	CHO	PLA
CHO, g/100 mL	11.48	15.15	0
PRO, g/100 mL	3.67	0	0
Fat, g/100 mL	2.05	2.05	0
kcals/100 mL	79.05	79.05	0
Ratio of CHO:PRO	3.12:1	--	--

Per 100 mL. CM, chocolate milk; CHO, carbohydrate + fat; PLA, placebo.

Table 5.3. Energy and macronutrient content of supplements

	Baseline	Midpoint	End
LT (VO_2, L/min)			
CM	1.61 ± 0.16	--	1.83 ± 0.16 [b]
CHO	1.47 ± 0.10	--	1.67 ± 0.11 [b]
PLA	1.53 ± 0.11	--	1.70 ± 0.13 [b]

Values are mean ± SE. Significant differences: [b] time only (P<0.05).

Table 5.4. Lactate threshold

	TT 2	TT 3	TT 4
TT Time (min)			
CM	53.4 ± 1.2	52.4 ± 1.5	51.8 ± 1.5 [b]
CHO	54.8 ± 1.6	52.8 ± 1.3	51.1 ± 1.2 [b]
PLA	59.1 ± 2.0	56.3 ± 2.2	54.7 ± 2.2 [b]
Power (Avg Watts)			
CM	143.1 ± 10.3	148.9 ± 12.8	152.6 ± 13.1 [b]
CHO	135.8 ± 10.3	143.3 ± 10.9	155.2 ± 10.0 [b]
PLA	126.5 ± 10.0	138.9 ± 11.2	143.2 ± 13.8 [b]

Values are mean ± SE. Significant differences: [b] time only (P<0.05).

Table 5.5. Time trial performance

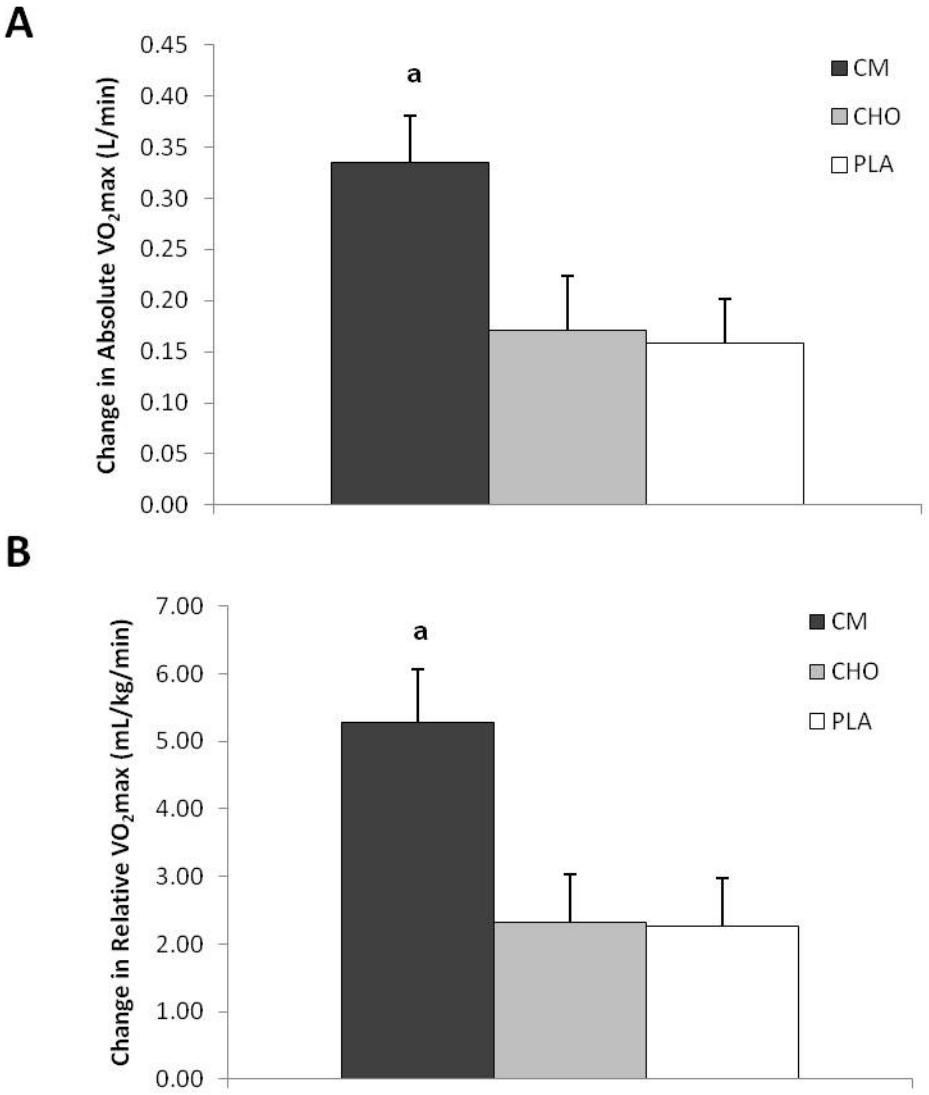

Figure 5.1. VO_2max changes after 4.5 wks of aerobic endurance training. A. Change from baseline in absolute VO_2max. **B.** Change from baseline in relative VO_2max. Values are mean ± SE. Significant treatment differences: [a] CM vs PLA and CHO (P<0.05).

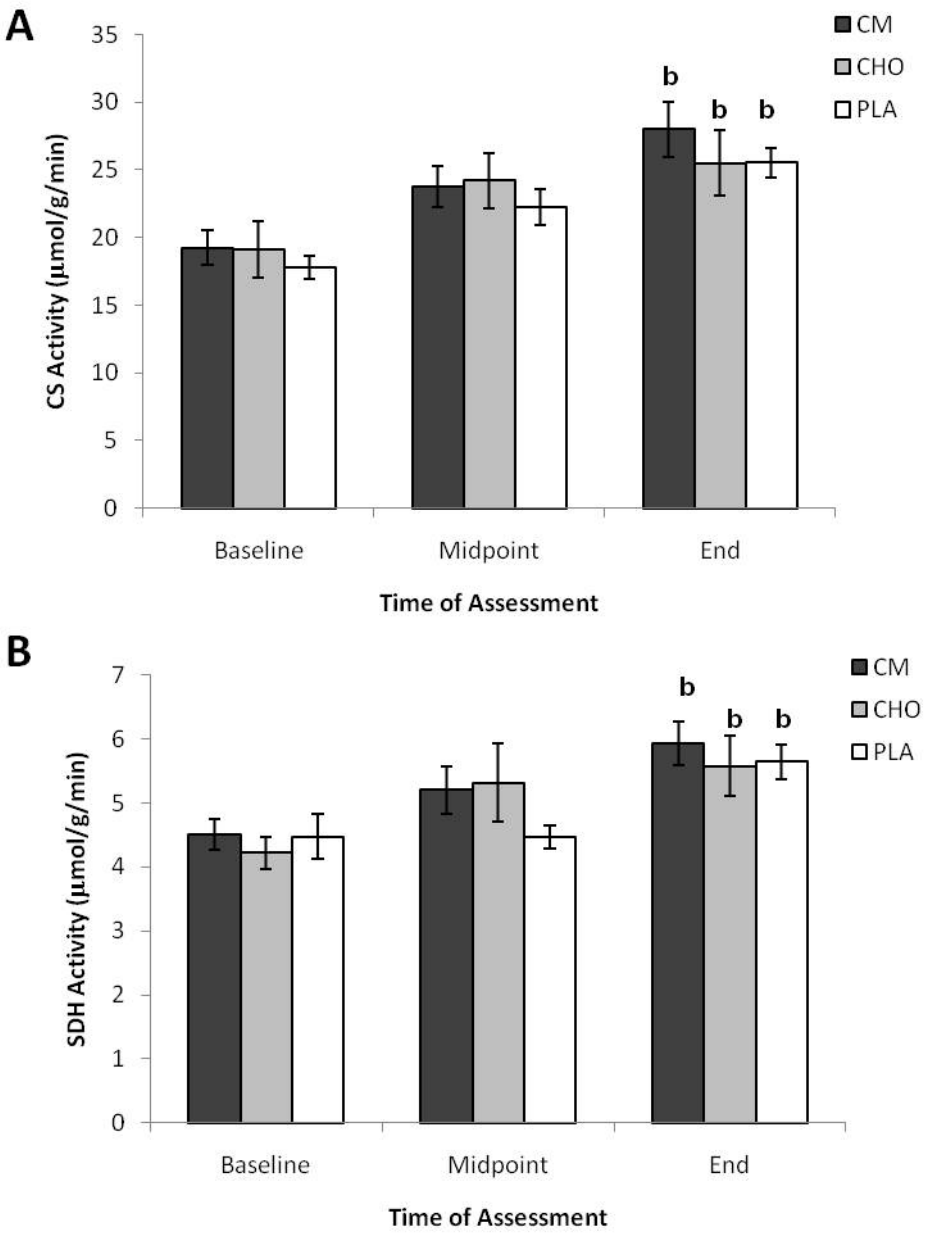

Figure 5.2. Oxidative enzyme activity. A. Citrate synthase activity. **B.** Succinate dehydrogenase activity. Biopsies were taken at baseline (before starting the training period), at the midpoint and at the end of the 4.5 wk training period. No significant treatment differences were found. [b] significant time effect ($P<0.05$).

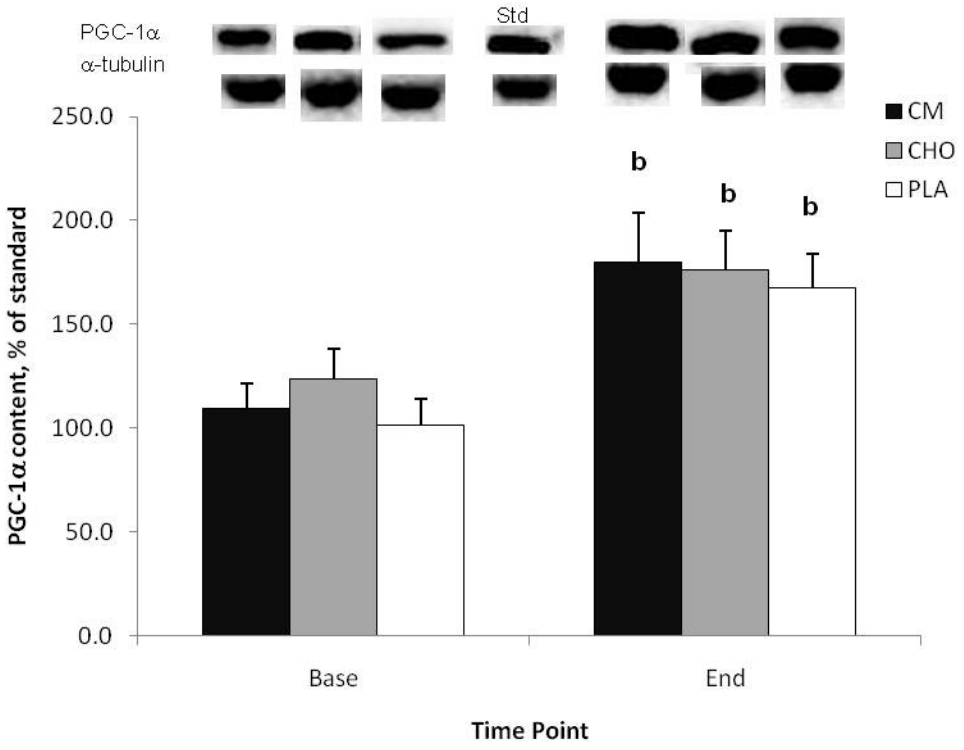

Figure 5.3. PGC-1α content before and after 4.5 wks of cycling exercise training. No significant treatment differences were found. [b] significant time effect ($P<0.05$).

Chapter VI: Effects of Aerobic Exercise Training and Supplementation on Body Composition, Immune Cells, Inflammatory Markers, and Metabolic Hormones

ABSTRACT

Investigations of resistance training have demonstrated body composition improvements when a CHO+PRO supplement is ingested post-exercise compared to CHO. However, no investigation has examined the effects of CHO+PRO supplementation in a model of aerobic endurance exercise training on body composition changes as well as markers of metabolic health. The purpose of the present study was to compare the effects of post-exercise CHO+PRO supplementation in the form of chocolate milk (CM), isocaloric carbohydrate (CHO), and placebo (PLA) on body composition changes and markers of immune system stress, inflammation and metabolic homeostasis that occurred in response to a 4.5 week aerobic exercise training program in healthy, untrained subjects. Thirty-two males and females were randomized into one of the 3 treatment groups. Subjects cycled for 1 h/d, 5 d/wk for 4.5 wks at 75-80% of VO_2max. Supplements were ingested immediately and 1 h after each session. Body composition analysis and fasting blood collections were performed at baseline, midpoint, and the end of the training period. Pre-and post-exercise blood samples were taken weekly. Greater improvements in body composition, represented by a calculated lean and fat mass differential for whole body and trunk region, were found in the CM group compared to CHO. No significant differences were found between baseline, midpoint and end time points for fasting insulin, cortisol, cytokines or immune cell counts. These results suggest that ingesting a CM supplement post-exercise during 4.5 wks of aerobic exercise training can improve body composition more effectively than isocaloric CHO or placebo.

INTRODUCTION

The importance of regular physical activity for improving or maintaining overall health has been well established. The cardiovascaular benefits of exercise are well documented (6, 26, 39, 40), and increasing evidence suggests that the possible anti-inflammatory effects of aerobic exercise may underlie the cardioprotective effects (14, 24, 43, 47). Regular exercise is also associated with metabolic improvements, such as in insulin sensitivity and glucose metabolism (22, 25). In addition, participating in regular physical activity is inversely associated with risk of developing an upper respiratory tract infection (10), although many investigations have reported little or no effect of exercise training on immune function (2, 7, 28) despite some alterations in circulating immune cell levels (28). In addition to participating in regular exercise to improve or maintain overall health, exercise training is often undertaken as a means to facilitate weight loss and improve body composition.

The beneficial effects of post-exercise supplementation in the form of carbohydrate (CHO) or carbohydrate-protein (CHO+PRO) in promoting recovery from, and adaptation to, acute exercise has been demonstrated (3, 19, 46, 53, 54). Others have reported the efficacy of milk-based supplements in increasing protein synthesis (11) and lean mass accrual (15, 21, 52) in response to resistance exercise. Recently, chocolate milk (CM) has been investigated as a practical and effective CHO+PRO post-exercise recovery supplement after aerobic exercise (23, 41, 51.). However, the effects of aerobic endurance exercise training and nutritional supplementation on body composition changes, as well as other markers of health such as immune system changes, inflammatory markers, plasma glucose, and metabolic hormones, have not been investigated.

Therefore, the purpose of the present study was to determine the effects of a 4.5 wk aerobic endurance exercise (cycling) training program when supplementing after each daily exercise session with a carbohydrate and protein containing beverage (chocolate milk, CM), carbohydrate only (CHO) or placebo. We aimed to determine if

supplementation resulted in a greater increase in lean mass, and a greater decrease in fat mass. We also sought to determine if immune cell levels were better maintained, and inflammatory markers better attenuated, in the CM group compared to the CHO or PLA groups. We hypothesized that the CM group would demonstrate greater lean body mass increases and fat mass decreases, and less immune cell and inflammatory cytokine perturbations during aerobic exercise training compared to the CHO and PLA groups.

METHODS

Subjects. Thirty-two healthy, recreationally active but untrained males and females (16 males and 16 females) between 18 and 35 years old completed the study. Subject characteristics are listed in Table 6.1. In order to be classified as recreationally active but not endurance trained, subjects could not have exercised regularly more than 3 h/wk over the last 2 years, and had VO_2max values of <40 mL/kg/min for females and <45 mL/kg/min for males. Potential subjects who did not meet these criteria were screened out of the study. A total of 36 subjects were admitted to the study, and 4 subjects voluntarily withdrew due to illness or work scheduling conflicts. Ten to twelve subjects per group were matched for age, gender and body composition, and then the subjects were randomized into one of the 3 treatment groups for the duration of the training period. Written informed consent was obtained from all subjects, and the study was approved by The University of Texas at Austin Institutional Review Board. Of the 32 subjects who completed the entire study, two subjects were excluded from blood analyses due to extreme difficulties in collecting blood from the antecubital veins, and two subjects were also excluded from body composition analysis due to exclusion criteria for DEXA scans.

Research design and experimental protocol. This study followed a randomized, double-blinded, placebo controlled design. The protocol for the training period is shown in Table 6.2. The entire protocol period was 7 wk long and consisted of the following: a baseline testing week; the first and second weeks of training; a midpoint testing week with 2 days of training; training weeks 3 and 4; and a partial week during which the end

testing was performed. Subjects reported to the laboratory before the start of their training period on two occasions, once for a baseline blood collection and DEXA scan for body composition determination (described below), and again the following day for determination of maximal oxygen consumption (VO_2max) and maximal workload (W_{max}). The blood collections were performed again at the midpoint of the study and at the end. DEXA scans were repeated at the end of the training period.

The VO_2max/W_{max} test was performed on a VeloTron DynaFit Pro cycle ergometer (RacerMate, Seattle, WA). VO_2max was measured using a TrueOne2400 system (ParvoMedics, Sandy, UT). Subjects breathed through a Hans Rudolph valve, with expired gases directed to a mixing chamber for analysis of oxygen (O_2) and carbon dioxide (CO_2). Outputs were directed to a computer for calculation of ventilation, O_2 consumption (VO_2), CO_2 production (VCO_2), and respiratory exchange ratio (RER) every 15 s. Subjects cycled at 125 W for males or 75 W for females for 4 min as a warm-up before beginning the VO_2max test. The protocol for establishing VO_2max consisted of 2 min stages beginning at 125 W for males or 75 W for females. The work load was increased by 50 W (males) or 30 W (females) every 2 min until 275 W and 200 W, respectively. After this point, the workload increased 25 W (males) or 20 W (females) every minute until the subject could not continue to pedal despite constant verbal encouragement. The criteria used to establish VO_2max was a plateau in VO_2 with increasing exercise intensity and RER > 1.10.

Maximum power output in Watts was calculated from the VO_2max test data using the formula, adapted from Astrand and Rodahl (1):

$$W_{max} = (VO_2max\ mL - 300\ mL\ O_2) / 12.5\ W/mL\ O_2$$

The workload for the desired intensity level of the training rides (75% of VO_2max for the first 3.5 weeks and 80% for week 4) was then set as percentages of the W_{max} as follows:

$$W = [(VO_2max\ mL \times \%VO_2max\ desired) - 300\ mL\ O_2] / 12.5\ W/mL\ O_2$$

During the training weeks, subjects reported to the training laboratory each morning after fasting overnight. All subjects began the rides as a group at the same time each day (6:00 AM or 7:30 AM), Monday – Friday. On Fridays, the subjects performed a cycling time trial (TT) that was designed to take about 1 h to complete. A pre- and post-exercise blood collection was taken for TTs 2, 3, and 4. After each session including the TTs, subjects were provided one dose of supplement immediately post-exercise and were required to drink it in the laboratory. The beverages and doses provided are described in detail below. Subjects were then provided a second dose in an opaque to-go cup with a lid and straw and instructed to drink it 1 h later. They were also instructed to not ingest anything other than water until 1 h after ingesting the second dose.

The daily training rides and time trials were performed on Kona Dew bicycles (Kona, Ferndale, WA) mounted on CompuTrainer stationary trainers (RacerMate, Seattle, WA) interfaced with MultiRider III software (RacerMate, Seattle, WA). Six bicycles and CompuTrainers were interfaced with the system to allow for training groups of 6 subjects at one time. The bikes were set up based on each subject's physical measurements. The CompuTrainers were calibrated each morning. To minimize thermal stress, air was circulated over the subjects with standing floor fans, and water was provided ad libitum. Investigators encouraged the subjects to drink as needed.

The first week of training served to get the subjects accustomed to cycling for prolonged periods. The first ride was 30 min in duration, the second was 40 min, the third ride, 50 min, and the fourth, 60 min. The first TT was a shortened version (16 km) of the 24-km TT course that was performed the next 3 wk and served to familiarize them with the concept of time trialing, while providing a shorter, more intense session. With the

exception of 3 rides the first week, all rides on Monday – Thursday were 1 h in duration throughout the training period. The Friday TTs were ~50-70 min in duration.

Each training ride and TT began with a 10-min warm up at 60% VO_2max, after which the work rate was increased to elicit ~75% VO_2max for duration of each training ride. At the midpoint, VO_2max was reassessed and the workloads were adjusted accordingly to keep the subjects exercising at 75% VO_2max for the third week. For the fourth week, the intensity was increased to 80% VO_2max. A 5-min VO_2 measurement was performed at the beginning of each week to verify that the workload corresponded to the calculated intensity (%VO_2max) for each subject. The Wattage calculated for each subject was set by the investigators, and subjects were asked to maintain a cadence of ~70 rpms in order to maintain the Wattage. Subjects were not allowed to shift gears or vary their cadence during the rides on Monday – Thursday. Each Friday of training weeks 2, 3, and 4, subjects performed a 25-km time trial, which took ~1 h or less to complete when riding at a high intensity. The subjects were allowed to shift gears on the bicycles in order to cycle the predetermined course as fast as possible, and were asked to ride the course as if it were a race.

(Data from the VO_2max tests and the TT data are presented in Study 3/Chapter V.)

Experimental beverages. After each daily session, subjects ingested the experimental beverages (CM, CHO, or PLA) immediately and l h post-exercise. The CM (Kirkland Organic Low-Fat Chocolate Milk, Costco Inc.) and CHO beverages were isocaloric and contained the same amount of fat. The placebo was an artificially flavored and artificially sweetened supplement that resembled the CHO beverage in taste and appearance, but contained no calories. The energy and macronutrient composition of the beverages is shown in Table 6.3.

The amounts of supplement provided were stratified according to body weight ranges. Subjects weighing less than 63.6 kg (140 lbs) received 250 mL per supplement (197.5 kcals each), totaling 500 mL and 395 kcals. Subjects weighing between 63.6 kg (140 lbs) and 77.2 kg (170 lbs) received 300 mL per supplement (237 kcals), totaling 600 mL and 474 kcals. Subjects weighing between 77.2 kg (170 lbs) and 90.9 kg (200 lbs) received 350 mL per supplement (277 kcals), totaling 700 mL and 554 kcals. Subjects weighing over 90.9 kg (200 lbs) received 375 mL per supplement (296.5 kcals), totaling 750 mL and 593 kcals. For the CHO treatment, the amount of carbohydrate (dextrose) and fat (canola oil) matched that provided in the CM as measured for the individual's weight range. Both the CHO and Placebo beverages were flavored with grape-flavored non-caloric Kool-Aid. The CM supplement provided an average of 0.94 g carbohydrate, 0.31 g protein, and 0.17 g fat per kg body weight. The CHO supplement provided an average of 1.25 g carbohydrate and 0.17 g fat per kg body weight.

Diet and exercise. Subjects were asked to keep their diets and activity levels consistent for the duration of the study (i.e.; no significant changes in caloric intake, dietary habits, or activity levels outside of the study's training sessions). The subjects were instructed to maintain a dietary and activity log for the 2 days prior to their baseline, midpoint and end biopsies and testing. They were also instructed to keep a 2-day dietary and activity log each week on Wednesdays and Thursdays before the Friday TT so that compliance in keeping their normal dietary habits and activity levels consistent prior to the TTs could be verified. In addition, the subjects were asked to replicate their diet and activity on the days the logs are kept, such that the diet and activity was the same on the 2 days prior to each TT or each blood collection session. Subjects were required to turn the logs in each week, and the logs were analyzed for macronutrient composition and total caloric intake using Nutritionist V Dietary Analysis Software (First Data Bank, Inc, San Bruno, CA). All subjects complied with the diet and activity requirements. Subjects were instructed to arrive at the laboratory having fasted overnight for 12 h for every exercise session and laboratory visit.

Blood sampling and analyses. Blood collections occurred at 6 time points during the training protocol, as shown in Table 6.2. All blood collections were performed by venipunctures in an antecubital vein with a 10 mL syringe. For baseline, midpoint and end blood collections, 5 mL of the total blood sample was mixed with 0.5 mL of EDTA (24 mg/mL, pH 7.4), and 0.3 mL of the anticoagulated blood was transferred to another tube containing 0.6 mL 10% perchloric acid (PCA). The remaining 5 mL was transferred directly into a K_2EDTA-coated Vacutainer tube for the white blood cell count with differentiation, performed by a clinical hematology laboratory (LabCorp, Austin, TX). The tubes were centrifuged at 4˚ C for 10 min at 3,000 rpm with a HS-4 rotor in a Sorvall RC6 centrifuge (Kendro Laboratory Products, Newtown, CT). After centrifugation, plasma and PCA extracts were separated into aliquots for each assay and immediately frozen and stored at -80˚C for later analysis. For the pre- and post-exercise collections that occurred for the time trials during weeks 2 and 4, 10 mL sample was collected and processed in the same manner as described above. For the week 3 TT, only 5 mLs were collected and no white blood cell analyses were performed.

Plasma glucose was measured using a spectophotometric Trinder reaction (no. 315, Sigma Chemical, St. Louis, MO) and had a CV of 3.7%. Plasma insulin was measured using ImmuChem^{TM} Coated Tube ^{125}I RIA Kit (MP Biomedicals, LLC, Orangeburg, NY) by radioimmunoassay. All samples were run in duplicate, with a coefficient of variation (CV) of 6.0%. A fasting glucose-to-insulin ratio was calculated from the plasma glucose and insulin values [(glucose, mg/dL)/insulin, μIU/mL)] at baseline, midpoint and end as described by Legro and colleagues (27), with values <4.5 being indicative of decreased insulin sensitivity (27). Blood lactate was determined from the PCA extract by enzymatic-spectophotometric analysis method based on the oxidation of lactate to pyruvate by nicotinamide adenine dicnucleotide (NAD^+)· (17), and had a CV of 1.5%. Plasma cortisol was measured using ImmuChem^{TM} Coated Tube ^{125}I RIA Kit (MP Biomedicals, LLC, Orangeburg, NY) by radioimmunoassay, with a CV of 6.1%. Total

plasma concentrations of interleukin-1 receptor antagonist (IL-1ra), interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-10 (IL-10), interleukin-1 beta (IL-1B), and tumor necrosis factor alpha (TNFα) were determined using Millipore High Sensitivity Multiplex Human Cytokine Assay kits (Millipore, Billerica, MA) with a Bio-Plex 200 multiplex suspension array system with Luminex xMAP detection technology (Luminex Corp., Austin, TX). All assays were run in duplicate.

Routine complete blood counts (CBC) with differentiation for total leukocytes and subsets (lymphocytes, neutrophils, and monocytes) were performed on 5 mLs of the blood samples collected at baseline, midpoint, end and pre- and post-exercise for TTs 2 and 4 as described above. The samples were transported on ice the same day as the collections occurred to a local clinical hematology laboratory (LabCorp, Austin, TX).

Body composition. Dual Energy X-Ray Absorbency (Medical Systems Prodigy, General Electric, Madison, WI) was used to determine both whole body and regional (trunk and legs) changes in fat mass and lean mass, as well as bone mineral density (BMD). A three-compartment model design for assessing body composition was used, dividing the body into bone, fat mass, and fat-free mass. The total region percentage of fat mass and lean mass were used to assess the subjects' body fat and lean mass levels. The trunk region and legs region were used to assess fat and lean mass changes in the trunk and legs independently. The DEXA machine was calibrated each morning prior to subject measurement. Measurements were performed at baseline and at the end of the training period on the same day as the blood collections were performed. The same trained technician performed all of the DEXA scans for the entire study.

The body composition differentials (Figure 6.1A-C) were calculated according to the formula:

$$(LMkg_{End} - LMkg_{Baseline}) - (FMkg_{End} - FMkg_{Baseline}) = \text{Differential (kg)}$$

Using this formula, a gain in lean mass and a loss of fat mass would result in a higher differential value than a loss in lean mass and gain in fat mass, or no change in lean and fat mass. This differential was calculated for whole body as well as regional (trunk and legs) changes (Figure 6.1A-C). Therefore, the whole body differential was calculated as follows, using the CM treatment group values as an example:

$$1.408 \text{ kg} - (-1.363 \text{ kg}) = 2.771 \text{ kg}$$

The regional differentials were calculated by the same formula using the values from those specific regions.

Statistical analyses. Absolute changes from baseline to end in the body composition differentials were analyzed using a one-way ANOVA. Differences in baseline, midpoint and end blood and plasma measures were analyzed using two-way (treatment x time) analysis of variance (ANOVA). Differences in pre- and post-TT blood and plasma measures for TT2 and TT4 were analyzed using two-way (treatment x time) ANOVA, while the absolute change in each TT (TTΔ) was analyzed using a one-way ANOVA. Differences in the TT2 absolute change compared to TT4 absolute change (TT2Δ vs TT4Δ) were analyzed using a two-way (treatment x time) ANOVA. For all measures, post hoc analysis was performed when significance was found using Least Significant Difference (LSD). Differences were considered significant at $P<0.05$. Data were expressed as mean ± SE. All statistical analyses were performed using SPSS version 16.0 statistical software (SPSS Inc., Chicago, IL).

RESULTS

Body composition. Changes in body weight, lean mass and fat mass (assessed for whole body, trunk, and legs) are shown in Table 6.4. Whole-body lean mass increased in all treatment groups, with no treatment differences detected ($P<0.05$). Although whole-body

fat mass decreased in all groups, the change was not significant for treatment or time. In the trunk region, a significantly greater gain in lean mass was found in the CM group compared to PLA (P<0.05). Trunk region fat mass differences were not significantly different between treatments, although a significant time effect was found for all groups (P<0.05). In the legs region, significant time effects were found for lean mass increases and fat mass decreases in all groups (P<0.05).

The whole body and regional differentials are shown in Figure 6.1A-C. The whole body differential and the trunk differential was significantly greater in the CM group compared to CHO (P<0.05). Whole body and trunk differentials for PLA were not significantly different than those for CM or CHO. The differential for the legs region was not significantly different among the three treatments. No significant treatment or time differences existed for BMD (Table 6.4).

Baseline, midpoint and end plasma analyses. No treatment or time effects were found for plasma insulin, cortisol, or cytokine levels (Table 6.5). Plasma glucose was significantly higher at the end of the training period in the PLA group compared to CM and CHO (Table 6.5) (P<0.05). No significant treatment or time effect was found for the fasting glucose-to-insulin ratio (Table 6.5).

Pre- and post exercise blood and plasma analyses. No treatment or time differences were found for plasma glucose in the pre- to post-TT measures (Table 6.6). Blood lactate was significantly lower at the end of TT4 in PLA than CM or CHO (P<0.05). A significant time effect (pre- to post-exercise) existed for lactate in all treatments. No significant time effect was found for the pre- to post-exercise measures for plasma cortisol. However, a significant treatment difference existed for both TT2 and TT4 in the CM group, which demonstrated higher pre- and post-exercise cortisol levels than CHO or PLA (Table 6.6). Levels of cytokines IL-1B, IL-6, IL-8, and IL-10 rose significantly from pre- to post-exercise for each TT in all treatment groups (P<0.05). With the exception of

IL-6, no significant differences were found between the treatments. IL-6 was higher in TT4 in the PLA group compared to CHO and CM ($P<0.05$) (Table 6.6). No significant treatment differences were detected for the TTΔs in any of the blood or plasma measures.

Immune cell counts. Concentrations of leukocytes and subpopulations (lymphocytes, neutrophils, and monocytes) are shown in Table 6.7. No significant treatment or time differences existed for the baseline, midpoint and end measures. No significant treatment differences existed for any of the TT pre- and post-exercise measures. Significant pre- to post-exercise time effects were found for total while blood cells, lymphocytes, neutrophils, and monocytes in all treatments for both TTs (Table 6.7, $P<0.05$), but there were no treatment differences in the TTΔ analyses for any of the leukocytes or subpopulations.

DISCUSSION

In the present study, we aimed to determine the effects of 4.5 wks of aerobic endurance exercise and post-exercise nutritional supplementation on body composition changes, and changes in immune cells and inflammatory markers. The most important finding was that body composition improvements, represented by a calculated lean and fat mass differential, was significantly greater in the CM group than the CHO group. Compared to the CHO group, the CM group lost more fat mass and gained more lean mass measured in the whole body, as well as in the trunk region only ($P<0.05$). While these differentials were also greater for CM compared with PLA, the differences were not significant. This improvement in body composition with CM is in agreement with the findings of Josse and colleagues (21), who recently demonstrated significantly greater muscle mass accretion, fat mass loss and strength gains with milk supplementation compared to soy and CHO after a 12-wk resistance training program (21). However, the training in the present study was aerobic exercise only, and thus, the body composition improvements are remarkable given the relatively short length of the training period (4.5 wks), the lack

of a resistance training component, and the lack of any type of energy restriction or dietary intake control.

As shown in Table 6.4, all groups demonstrated significant changes over time in whole-body lean mass, trunk fat mass, and legs lean and fat mass, and the CM group demonstrated a significant treatment effect compared to CHO when whole body and regional differentials were calculated (Figure 6.1A-C). The whole body and trunk differentials for PLA were slightly greater than CHO, although not significantly different from either CM or CHO. The lack of difference in the PLA treatment from CHO suggests that a component of the CM treatment facilitated the significant body composition change; simply supplementing with an energy-containing supplement (CHO) did not have a significant effect compared to PLA. In fact, a slight, non-significant increase in fat mass in the legs region was detected with CHO, whereas fat mass of the legs decreased in CM and PLA during the training period. To our knowledge, no evidence exists in the literature to suggest that post-exercise CHO supplementation would mediate this type of change, given that the subjects' diets were not standardized and controlled during the study. However, this finding further underscores the difference in supplementing with a CHO+PRO-containing supplement versus calories from CHO alone in facilitating body composition changes.

There are two possible explanations for the difference found with the CM treatment compared to CHO: first, the availability of amino acids (AA) in the milk for anabolism and muscle mass accretion, and second, a fat-loss promoting effect of dairy calcium and protein. It is known that AA, along with a permissive amount of insulin, is required for muscle protein accretion to occur in response to exercise (4, 5). The CHO treatment would increase plasma insulin levels and provide glucose as an energy and glycogen-synthesizing substrate, but would provide no AA for the synthesis of new muscle protein. Thus, AA availability from the milk proteins whey and casein provided substrate for this adaptive process. In addition, Zemel and colleagues (55, 56) have shown that the

increased consumption of dietary calcium is associated with reduced adiposity and greater weight loss in energy restricted diets. Moreover, the fat and weight loss effects were greater when the source of the dietary calcium was from dairy products rather than a calcium supplement (55, 56). Additional evidence that the dairy component of the CM treatment likely underlies some of the body composition changes is found in the resistance training study of Hartman and colleagues (15), who demonstrated that fat mass decreased, and lean mass increased, in groups provided either milk, soy, or CHO post-exercise, but that milk significantly promoted increased hypertrophy compared to soy and CHO (15). Another well-known benefit of dairy calcium consumption is improved bone mineral density. We did not detect treatment or time differences in BMD (Table 6.4); however, this is not surprising, given the relatively short duration of the training program and the lack of a resistance training component. Taken together, these data suggest that the dairy component of the CM treatment was instrumental in facilitating the fat mass changes compared to the CHO and PLA groups, while the AA from milk proteins provided substrate for lean mass accretion in the present study.

As shown in Table 6.5, we found no significant treatment or time differences in any of the blood measures taken at baseline, midpoint and the end of the training period except in plasma glucose, which was higher at the end of the training period in the PLA group compared to CM or CHO, although still within a normal fasting range. While there were no significant differences in the baseline glucose values among the three treatments, the baseline values in PLA were slightly higher in this group than the CM or CHO groups, which led to the treatment difference. Plasma insulin was not different between the three groups, and there was no treatment or time difference for the fasting glucose-to-insulin ratio, which is an indicator of insulin sensitivity (27). It is known that those individuals who train regularly have greater glucose tolerance and a lowered insulin response to a glucose challenge than age and weight-matched sedentary individuals (18), and that fasting plasma glucose decreases in obese, insulin resistant individuals after 6-10 wks of aerobic training (44). Since the subjects in the present study were young, healthy, and not

obese or insulin resistant, it is not likely that an exercise or treatment effect in these measures would be detected.

Significant treatment differences were found for three analytes in the pre- to post-exercise measures in the present study: plasma IL-6, plasma cortisol, and blood lactate. Plasma IL-6 was found to be significantly higher post-exercise in the PLA group for TT4 compared to both CM and CHO, whereas plasma cortisol in the CM group was higher pre- and post-exercise in TT2 and TT4 than CHO or PLA (Table 6.6; $P<0.05$). IL-6 is often used as marker of inflammation because it is the first cytokine to appear in response to intense exercise, and demonstrates the largest measurable increase. This increase can reach up to 100-fold during exercise before declining in the post-exercise period (13, 36-38, 49). It is known that ingesting CHO during prolonged, intense exercise attenuates the increase in both pro- and anti-inflammatory cytokines, as well as the increase in cortisol, but the effects of training and post-exercise supplementation on the cytokine response after a fasted exercise bout, such as was performed in the present study, has not previously been characterized.

While it is difficult to make a general interpretation based on a single cytokine response, it is important to note that IL-6 has been implicated as having important roles in influencing glucose homeostasis during exercise by increasing endogenous glucose production (12). Therefore, it may be that the increased IL-6 post-exercise found in the PLA group is related to a possible reduction in muscle glycogen storage in the hours after the daily training rides. The significantly lower lactate response during exercise in the PLA group appears to support this hypothesis. In the final pre-post exercise assessment (TT4), the PLA group had a significantly lower lactate response to the bout compared to the CM and CHO groups. Taken together, these data suggest that the PLA group likely experienced diminished muscle glycogen resynthesis during the consecutive days of training, which may have affected the availability of glycogen during the intense exercise bout.

Ostrowski and colleagues (33) previously demonstrated that strenuous, prolonged running elicited a dramatic increase in IL-6, as well as an increase in the pro-inflammatory cytokines TNFα and IL-1B (32-34). They also found that this increase in pro-inflammatory cytokines was balanced by corresponding increases in cytokine inhibitors IL-1Ra and soluble TNFα receptors, as well as the anti-inflammatory cytokine IL-10 (33). IL-10 inhibits the release of TNFα and IL-1B (9), which may explain why these two classic pro-inflammatory cytokines often do not show a large significant increase in exercise. IL-10 also induces production of IL-1Ra (8, 20), which is the antagonist of the IL-1 receptor, again possibly explaining a lack of increase in IL-1B detection after exercise in many investigations. In the present study, however, IL-10 increased significantly in both TTs across all groups, but IL-1Ra did not. This lack of significant increase during exercise in IL-1Ra may explain the significant increase in IL-1B, which increased significantly pre- to post-exercise in both TTs in all treatment groups. This suggests that muscle damage and inflammation, evidenced by IL-1B elevations, was not influenced by treatment. While the pro-inflammatory cytokine TNFα increased during both TTs, this increase reached statistical significance only in TT4 and in all treatment groups. Therefore, the findings of the current study suggest that the post-exercise supplementation does not affect the inflammatory or muscle damage response to an acute, intense exercise bout performed in the fasted state. These results would likely be different if supplementation were provided during the bout itself.

The significantly higher plasma cortisol in the pre- and post-TT exercise cortisol values in the CM group compared to the CHO group in TT2, and compared to both the CHO and PLA groups in TT4, is likely due to the slightly higher pre-TT (resting) levels. While no statistically significant treatment or time differences existed in the baseline, midpoint or end fasting cortisol measures, the CM group had higher plasma cortisol levels at all three time points than CHO or PLA. Therefore, the elevated exercise values for cortisol in the CM group is primarily due to higher resting values in this group compared to CHO and

PLA. In moderately vigorous exercise of 1 h, such as occurred in the present study, cortisol normally does not increase significantly. Typically, it comes into play in prolonged bouts, and exerts its effect hours after exercise ceases (36). Due to that lag-time effect, cortisol is most likely responsible for maintaining the lymphocytopenia and neutrophilia normally seen after prolonged exercise (35). Despite the known effects on immune cell function and concentrations, especially on neutrophil levels and function (16, 48, 50), no treatment group differences existed for the immune cell counts, and the increases from pre- to post-exercise found in the leukocyte subpopulations are typical of the exercise response in these cells (29-31, 36).

It is important to note that in the present investigation, we did not test immune function directly with measures such as lymphocyte proliferation or oxidative burst activity, but rather used resting and pre/post exercise immune cell concentration changes as an indicator of the immune response to the training and supplementation. In a previous study of cycling exercise training on immune function, Rhind and colleagues (45) had sedentary subjects perform 12 wk of cycling training at 65-70% VO_2max for 30 min/d, 4-5 d/wk (45). An acute bout of exercise (1 h at 60% VO_2max) was performed before and after the training period, and blood was collected at rest and after exercise. Compared to baseline and control measures, the individuals exhibited a larger increase in the natural killer (NK) cell count, reduced lymphocytopenia and attenuation of exercise-induced suppression of lymphocyte proliferation after the training period (45), which suggests the possibility for improved immune system function. In the present study, we did not observe a significant treatment or time difference in the resting nor the pre/post exercise lymphocyte counts after training for 4.5 wks. It may be that the training program length was not long enough to sufficiently impact the immune system, and therefore detect a change in the cell counts. We also did not assess NK cell concentrations or activity, which may have yielded a better indication of immune system function or stress. At this time, we can conclude that 4.5 wks of cycling training for 1 h/d, 5 d/wk at ~75% VO_2max

appears to have no deleterious or advantageous effects on immune cell concentrations at rest and in response to an acute, intense exercise bout.

In summary, CM supplementation significantly improved body composition as defined by the combination of an increase in lean mass and a decrease in fat mass compared to CHO in response to a 4.5 wk aerobic exercise training program. In addition, these results also suggest that 4.5 wks of aerobic exercise training does not appear to perturb resting immune cell concentrations or most markers of inflammation, although no differences existed between the energy-containing treatments (CM and CHO) and exercise only (PLA). Therefore, we conclude that ingesting a CM supplement post-exercise during 4.5 wks of aerobic exercise training can improve body composition more effectively than isocaloric CHO or placebo.

REFERENCES

1. Åstrand PO, and Rodahl K. Textbook of work physiology: Physiological bases of exercise. . New York: McGraw-Hill Book Company, 1977.
2. Baslund B, Lyngberg K, Andersen V, Halkjaer Kristensen J, Hansen M, Klokker M, and Pedersen BK. Effect of 8 wk of bicycle training on the immune system of patients with rheumatoid arthritis. J Appl Physiol 75: 1691-1695, 1993.
3. Baty JJ, Hwang H, Ding Z, Bernard JR, Wang B, Kwon B, and Ivy JL. The effect of a carbohydrate and protein supplement on resistance exercise performance, hormonal response, and muscle damage. J Strength Cond Res 21: 321–329, 2007.
4. Biolo G, Tipton KD, Klein S, and Wolfe RR. An abundant supply of amino acids enhances the metabolic effect of exercise on muscle protein. Am J Physiol Endocrinol Metab 273: E122-129, 1997.
5. Biolo G, Williams BD, Fleming RY, and Wolfe RR. Insulin action on muscle protein kinetics and amino acid transport during recovery after resistance exercise. Diabetes 48: 949-957, 1999.
6. Bowles DK, Woodman CR, and Laughlin MH. Coronary smooth muscle and endothelial adaptations to exercise training. Exerc Sport Sci Rev 28: 57-62, 2000.
7. Campbell PT, Wener MH, Sorensen B, Wood B, Chen-Levy Z, Potter JD, McTiernan A, and Ulrich CM. Effect of exercise on in vitro immune function: a 12-month randomized, controlled trial among postmenopausal women. J Appl Physiol 104: 1648-1655, 2008.
8. Cassatella MA, Meda L, Gasperini S, Calzetti F, and Bonora S. Interleukin 10 (IL-10) upregulates IL-1 receptor antagonist production from lipopolysaccharide-stimulated human polymorphonuclear leukocytes by delaying mRNA degradation. J Exp Med 179: 1695-1699, 1994.
9. Chernoff AE, Granowitz EV, Shapiro L, Vannier E, Lonnemann G, Angel JB, Kennedy JS, Rabson AR, Wolff SM, and Dinarello CA. A randomized, controlled trial of IL-10 in humans. Inhibition of inflammatory cytokine production and immune responses. J Immunol 154: 5492-5499, 1995.
10. Chubak J, McTiernan A, Sorensen B, Wener MH, Yasui Y, Velasquez M, Wood B, Rajan KB, Wetmore CM, Potter JD, and Ulrich CM. Moderate-intensity exercise reduces the incidence of colds among postmenopausal women. Am J Med 119: 937-942, 2006.
11. Elliot TA, Cree MG, Sanford AP, Wolfe RR, and Tipton KD. Milk ingestion stimulates net muscle protein synthesis following resistance exercise. Med Sci Sports Exerc 38: 667-674, 2006.
12. Febbraio MA, Hiscock N, Sacchetti M, Fischer CP, and Pedersen BK. Interleukin-6 Is a Novel Factor Mediating Glucose Homeostasis During Skeletal Muscle Contraction. Diabetes 53: 1643-1648, 2004.
13. Febbraio MA, and Pedersen BK. Muscle-derived interleukin-6: mechanisms for activation and possible biological roles. FASEB J 16: 1335-1347, 2002.

14. Ford ES. Does exercise reduce inflammation? Physical activity and C-reactive protein among U.S. adults. Epidemiology 13: 561-568, 2002.
15. Hartman JW, Tang JE, Wilkinson SB, Tarnopolsky MA, Lawrence RL, Fullerton AV, and Phillips SM. Consumption of fat-free fluid milk after resistance exercise promotes greater lean mass accretion than does consumption of soy or carbohydrate in young, novice, male weightlifters. Am J Clinical Nutrition 86: 373-381, 2007.
16. Hoffman-Goetz L, and Pedersen BK. Exercise and the immune system: a model of the stress response? Immunol Today 15: 382-387, 1994.
17. Hohorst HJ. Determination of L-lactate with LDH and DPN. In: Methods of Enzymatic Analysis, edited by Bergmeyer HU. New York: Academic, 1963, p. 266-270.
18. Ivy JL. Role of exercise training in the prevention and treatment of insulin resistance and non-insulin-dependent diabetes mellitus. Sports Med 24: 321-336, 1997.
19. Ivy JL, Ding Z, Hwang H, Cialdella-Kam LC, and Morrison PJ. Post exercise carbohydrate-protein supplementation: phosphorylation of muscle proteins involved in glycogen synthesis and protein translation. Amino Acids 35: 89-97, 2008.
20. Jenkins JK, Malyak M, and Arend WP. The effects of interleukin-10 on interleukin-1 receptor antagonist and interleukin-1 beta production in human monocytes and neutrophils. Lymphokine Cytokine Res 13: 47-54, 1994.
21. Josse AR, Tang JE, Tarnopolsky MA, and Phillips SM. Body composition and strength changes in women with milk and resistance exercise. Med Sci Sports Exerc 42: 1122-1130, 2010.
22. Kahn SE, Larson VG, Beard JC, Cain KC, Fellingham GW, Schwartz RS, Veith RC, Stratton JR, Cerqueira MD, and Abrass IB. Effect of exercise on insulin action, glucose tolerance, and insulin secretion in aging. Am J Physiol Endocrinol Metab 258: E937-943, 1990.
23. Karp JR, Johnston JD, Tecklenburg S, Mickleborough TD, Fly AD, and Stager JM. Chocolate Milk as a Post-Exercise Recovery Aid. International Journal of Sport Nutrition and Exercise Metabolism 16: 78-91, 2006.
24. King DE, Carek P, Mainous AG, and Pearson WS. Inflammatory markers and exercise: differences related to exercise type. Med Sci Sports Exerc 35: 575-581, 2003.
25. Krotkiewski M, Lonnroth P, Mandroukas K, Wroblewski Z, Rebuffe-Scrive M, Holm G, Smith U, and Bjorntorp P. The effects of physical training on insulin secretion and effectiveness and on glucose metabolism in obesity and type 2 (non-insulin-dependent) diabetes mellitus. Diabetologia 28: 881-890, 1985.
26. Laughlin MH, and McAllister RM. Exercise training-induced coronary vascular adaptation. J Appl Physiol 73: 2209-2225, 1992.
27. Legro RS, Finegood D, and Dunaif A. A Fasting Glucose to Insulin Ratio Is a Useful Measure of Insulin Sensitivity in Women with Polycystic Ovary Syndrome. J Clin Endocrinol Metab 83: 2694-2698, 1998.
28. Nehlsen-Cannarell SL, Nieman DC, Balk-Lamberton AJ, Markoff PA, Chritton DB, Gusewitch G, and Lee JW. The effects of moderate exercise training on immune response. Med Sci Sports Exerc 23: 64-70, 1991.

29. Nieman DC. Exercise, infection, and immunity. Int J Sports Med 15 Suppl 3: S131-141, 1994.
30. Nieman DC. Exercise, upper respiratory tract infection, and the immune system. Med Sci Sports Exerc 26: 128-139, 1994.
31. Nieman DC, Henson DA, Austin MD, and Brown VA. Immune response to a 30-minute walk. Med Sci Sports Exerc 37: 57-62, 2005.
32. Ostrowski K, Hermann C, Bangash A, Schjerling P, Nielsen JN, and Pedersen BK. A trauma-like elevation of plasma cytokines in humans in response to treadmill running. J Physiol 513: 889-894, 1998.
33. Ostrowski K, Rohde T, Asp S, Schjerling P, and Pedersen BK. Pro- and anti-inflammatory cytokine balance in strenuous exercise in humans. J Physiol 515: 287-291, 1999.
34. Ostrowski K, Rohde T, Zacho M, Asp S, and Pedersen BK. Evidence that interleukin-6 is produced in human skeletal muscle during prolonged running. J Physiol 508: 949-953, 1998.
35. Pedersen BK, Bruunsgaard H, Klokker M, Kappel M, MacLean DA, Nielsen HB, Rohde T, Ullum H, and Zacho M. Exercise-induced immunomodulation--possible roles of neuroendocrine and metabolic factors. Int J Sports Med 18 Suppl 1: S2-7, 1997.
36. Pedersen BK, and Hoffman-Goetz L. Exercise and the Immune System: Regulation, Integration, and Adaptation. Physiol Rev 80: 1055-1081, 2000.
37. Pedersen BK, Steensberg A, and Schjerling P. Muscle-derived interleukin-6: possible biological effects. J Physiol 536: 329-337, 2001.
38. Petersen AMW, and Pedersen BK. The anti-inflammatory effect of exercise. J Appl Physiol 98: 1154-1162, 2005.
39. Powers SK, Demirel HA, Vincent HK, Coombes JS, Naito H, Hamilton KL, Shanely RA, and Jessup J. Exercise training improves myocardial tolerance to in vivo ischemia-reperfusion in the rat. Am J Physiol Regulatory Integrative Comp Physiol 275: R1468-1477, 1998.
40. Powers SK, Lennon SL, Quindry J, and Mehta JL. Exercise and cardioprotection. Curr Opin Cardiol 17: 495-502, 2002.
41. Pritchett K, Bishop P, Pritchett R, Green M, and Katica C. Acute effects of chocolate milk and a commercial recovery beverage on postexercise recovery indices and endurance cycling performance. Appl Physiol Nutr Metab 34: 1017-1022, 2009.
42. Rankin JW, Goldman LP, Puglisi MJ, Nickols-Richardson SM, Earthman CP, and Gwazdauskas FC. Effect of Post-Exercise Supplement Consumption on Adaptations to Resistance Training. J Am Coll Nutr 23: 322-330, 2004.
43. Rauramaa R, Halonen P, Vaisanen SB, Lakka TA, Schmidt-Trucksass A, Berg A, Penttila IM, Rankinen T, and Bouchard C. Effects of Aerobic Physical Exercise on Inflammation and Atherosclerosis in Men: The DNASCO Study: A Six-Year Randomized, Controlled Trial. Ann Intern Med 140: 1007-1014, 2004.
44. Reitman JS, Vasquez B, Klimes I, and Nagulesparan M. Improvement of glucose homeostasis after exercise training in non-insulin-dependent diabetes. Diabetes Care 7: 434-441, 1984.

45. Rhind SG, Shek PN, Shinkai S, and Shephard RJ. Effects of moderate endurance exercise and training on in vitro lymphocyte proliferation, interleukin-2 (IL-2) production, and IL-2 receptor expression. Eur J Appl Physiol Occup Physiol 74: 348-360, 1996.
46. Saunders M, Kane M, and Todd K. Effects of a Carbohydrate-Protein Beverage on Cycling Endurance and Muscle Damage. Med Sci Sports Exc 36: 1233-1238, 2004.
47. Sloan RP, Shapiro PA, DeMeersman RE, McKinley PS, Tracey KJ, Slavov I, Fang Y, and Flood PD. Aerobic exercise attenuates inducible TNF production in humans. J Appl Physiol 103: 1007-1011, 2007.
48. Suzuki K, Naganuma S, Totsuka M, Suzuki KJ, Mochizuki M, Shiraishi M, Nakaji S, and Sugawara K. Effects of exhaustive endurance exercise and its one-week daily repetition on neutrophil count and functional status in untrained men. Int J Sports Med 17: 205-212, 1996.
49. Suzuki K, Nakaji S, Yamada M, Totsuka M, Sato K, and Sugawara K. Systemic inflammatory response to exhaustive exercise. Cytokine kinetics. Exerc Immunol Rev 8: 6-48, 2002.
50. Suzuki K, Sato H, Kikuchi T, Abe T, Nakaji S, Sugawara K, Totsuka M, Sato K, and Yamaya K. Capacity of circulating neutrophils to produce reactive oxygen species after exhaustive exercise. J Appl Physiol 81: 1213-1222, 1996.
51. Thomas K, Morris P, and Stevenson E. Improved endurance capacity following chocolate milk consumption compared with 2 commercially available sport drinks. Appl Physiol Nutr Metab 34: 78-82, 2009.
52. Wilkinson SB, Tarnopolsky MA, MacDonald MJ, MacDonald JR, Armstrong D, and Phillips SM. Consumption of fluid skim milk promotes greater muscle protein accretion after resistance exercise than does consumption of an isonitrogenous and isoenergetic soy-protein beverage. Am J Clinical Nutrition 85: 1031-1040, 2007.
53. Williams MB, Raven PB, Fogt DL, and Ivy JL. Effects of Recovery Beverages on Glycogen Restoration and Endurance Exercise Performance. Journal of Strength and Conditioning Research 17: 12-19, 2003.
54. Zawadzki KM, Yaspelkis BB, 3rd, and Ivy JL. Carbohydrate-protein complex increases the rate of muscle glycogen storage after exercise. J Appl Physiol 72: 1854-1859, 1992.
55. Zemel MB. Mechanisms of Dairy Modulation of Adiposity. J Nutr 133: 252S-256, 2003.
56. Zemel MB. Role of calcium and dairy products in energy partitioning and weight management. Am J Clinical Nutrition 79: 907S-912, 2004.

	All subjects (32)	CM (11)	CHO (11)	PLA (10)
Age (y)	22.0 ± 0.5	22.1 ± 0.7	21.3 ± 0.9	22.6 ± 1.0
Weight (kg)	71.7 ± 2.4	70.9 ± 5.1	71.2 ± 3.1	73.2 ± 4.5
Height (cm)	168.6 ± 1.5	169.1 ± 2.3	168.0 ± 2.7	168.8 ± 3.1
VO_2max ($L \cdot min^{-1}$)	2.6 ± 0.2	2.7 ± 0.3	2.6 ± 0.2	2.6 ± 0.2
VO_2max ($mL \cdot kg \cdot min^{-1}$)	35.9 ± 1.9	36.8 ± 1.4	35.7 ± 2.2	35.2 ± 2.1

Values are mean ± SE.

Table 6.1. Subject characteristics at baseline

	Mon	Tue	Wed	Thurs	Fri	Sat	Sun
Baseline	VO_2max testing, blood collection, CBC, DEXA						
Week 1 (75% VO_2max)	30 min	40 min	50 min	60 min	TT	Rest	Rest
Week 2 (75% VO_2max)	60 min	60 min	60 min	60 min	TT/blood	Rest	Rest
Midpoint	Blood collection, VO_2max testing, CBC, DEXA			60 min	60 min	Rest	Rest
Week 3 (75% VO_2max)	60 min	60 min	60 min	60 min	TT/blood	Rest	Rest
Week 4 (80% VO_2max)	60 min	60 min	60 min	60 min	TT/blood	Rest	Rest
End	Blood collection, CBC, DEXA						

Table 6.2. Protocol for training period

	CM	CHO	PLA
CHO, g/100 mL	11.48	15.15	0
PRO, g/100 mL	3.67	0	0
Fat, g/100 mL	2.05	2.05	0
kcals/100 mL	79.05	79.05	0
Ratio of CHO:PRO	3.12:1	--	--

Per 100 mL. CM, chocolate milk; CHO, carbohydrate + fat; PLA, placebo.

Table 6.3. Energy and macronutrient content of supplements

	Baseline	End
Weight (kg)		
CM	71.7 ± 5.5	71.7 .± 5.5
CHO	71.4 ± 3.4	71.4 ± 3.4
PLA	73.2 ± 4.5	72.9 ± 4.4
Lean mass, whole body (kg)		
CM	49.56 ± 4.10	50.96 ± 4.07 [a]
CHO	49.42 ± 3.74	50.06 ± 3.74 [a]
PLA	47.73 ± 3.69	48.51 ± 3.55 [a]
Fat mass, whole body (kg)		
CM	19.09 ± 2.18	17.72 ± 2.15
CHO	19.03 ± 2.13	18.46 ± 1.91
PLA	22.54 ± 2.57	21.54 ± 2.57
Lean mass, trunk (kg)		
CM	23.67 ± 1.78	24.57 ± 1.67 [c]
CHO	22.60 ± 1.69	22.74 ± 1.67
PLA	20.92 ± 1.64	21.32 ± 1.58
Fat mass, trunk (kg)		
CM	11.61 ± 1.60	10.75 ± 1.61 [a]
CHO	10.16 ± 1.26	9.59 ± 1.06 [a]
PLA	10.70 ± 1.32	10.04 ± 1.27 [a]
Lean mass, legs (kg)		
CM	17.85 ± 1.41	18.27 ± 1.39 [a]
CHO	16.68 ± 1.27	17.14 ± 1.32 [a]
PLA	15.64 ± 1.21	15.98 ± 1.16 [a]
Fat mass, legs (kg)		
CM	6.96 ± 0.89	6.57 ± 0.90 [a]
CHO	6.74 ± 0.79	6.80 ± 0.81 [a]
PLA	7.72 ± 0.83	7.49 ± 0.82 [a]
Bone mineral density (g/cm^2)		
CM	1.24 ± 0.04	1.24 ± 0.05
CHO	1.21 ± 0.04	1.21 ± 0.04
PLA	1.18 ± 0.02	1.18 ± 0.02

Values are mean ± SE. Significant differences: [a] significant time effect; [c] CM vs CHO *(P*<0.05).

Table 6.4. Body composition

	Baseline	Midpoint	End
Glucose (mmol/L)			
CM	4.32 ± 0.14	4.22 ± 0.13	4.20 ± 0.10
CHO	4.20 ± 0.22	4.13 ± 0.16	4.20 ± 0.17
PLA	4.55 ± 0.11	4.40 ± 0.12	4.66 ± 0.19 [d]
Insulin (pmol/L)			
CM	102.7 ± 9.1	104.1 ± 10.3	101.3 ± 7.0
CHO	119.8 ± 12.6	115.4 ± 16.5	123.4 ± 16.7
PLA	127.8 ± 13.6	112.2 ± 19.1	129.5 ± 28.9
Glucose-insulin ratio			
CM	5.8 ± 0.6	5.6 ± 0.6	5.5 ± 0.4
CHO	4.8 ± 0.5	5.3 ± 0.7	5.1 ± 0.7
PLA	4.8 ± 0.5	5.7 ± 0.6	5.4 ± 0.6
Cortisol (nmol/L)			
CM	1047.6 ± 157.9	1264 ± 228.7	1082 ± 186.3
CHO	953.3 ± 62.0	860.3 ± 47.5	825.0 ± 61.8
PLA	837.8 ± 105.3	909.8 ± 103.9	898.0 ± 95.3
IL-1B (pg/mL)			
CM	2.06 ± 0.72	2.22 ± 1.55	2.77 ± 1.71
CHO	11.60 ± 5.17	9.45 ± 6.03	6.26 ± 2.42
PLA	6.11 ± 2.35	5.18 ± 1.79	6.98 ± 2.95
IL-6 (pg/mL)			
CM	3.36 ± 1.73	3.43 ± 1.96	3.56 ± 1.93
CHO	8.23 ± 5.79	2.98 ± 1.95	8.52 ± 5.93
PLA	11.16 ± 4.46	15.85 ± 7.90	17.23 ± 7.51
IL-8 (pg/mL)			
CM	6.19 ± 1.56	5.30 ± 1.53	5.05 ± 1.52
CHO	15.73 ± 9.15	10.11 ± 6.35	13.39 ± 6.49
PLA	19.93 ± 8.63	18.40 ± 6.15	28.49 ± 17.42
IL-10 (pg/mL)			
CM	4.49 ± 2.11	4.67 ± 3.19	4.71 ± 3.07
CHO	4.39 ± 1.85	1.78 ± 0.85	2.45 ± 1.06
PLA	9.94 ± 7.84	10.28 ± 6.78	9.63 ± 7.43
IL-1Ra (pg/mL)			
CM	1.33 ± 0.81	7.39 ± 5.36	3.24 ± 2.10
CHO	1.73 ± 0.70	1.90 ± 1.41	1.59 ± 1.23
PLA	9.88 ± 6.60	7.51 ± 4.98	8.37 ± 5.62
TNFα (pg/mL)			
CM	9.92 ± 3.83	11.52 ± 4.85	8.77 ± 3.26
CHO	8.43 ± 1.71	8.38 ± 1.76	9.48 ± 2.63
PLA	13.90 ± 6.11	10.80 ± 2.28	15.86 ± 5.11

Values are mean ± SE. Significant difference: [d] PLA vs CM and CHO (P<0.05).

Table 6.5. Baseline, midpoint and end blood and plasma measures

	TT2 Pre	TT2 Post	TT2Δ	TT 4 Pre	TT 4 Post	TT4Δ
Glucose (mmol/L)						
CM	4.17 ± 0.11	4.15 ± 0.17	-0.02 ± 0.6	4.26 ± 0.12	4.34 ± 0.22	0.08 ± 0.10
CHO	4.05 ± 0.14	4.22 ± 0.24	0.17 ± 10.0	4.11 ± 0.18	4.42 ± 0.31	0.31 ± 0.17
PLA	4.41 ± 0.14	4.35 ± 0.22	-0.06 ± 0.8	4.55 ± 0.12	4.64 ± 0.22	0.09 ± 0.10
Lactate (mmol/L)						
CM	0.88 ± 0.07	5.53 ± 0.80 [a]	4.65 ± 0.73	0.90 ± 0.07	5.15 ± 0.83 [a]	4.25 ± 0.76
CHO	1.00 ± 0.20	4.76 ± 0.76 [a]	3.76 ± 0.56	0.78 ± 0.04	6.37 ± 0.80 [a]	5.59 ± 0.76
PLA	0.99 ± 0.24	4.33 ± 0.73 [a]	3.34 ± 0.49	0.78 ± 0.08	3.84 ± 0.73 [a e]	3.06 ± 0.65
Cortisol (pmol/L)						
CM	1137 ± 211.8	1289.0 ± 210.6 [c]	152.0 ± 1.2	1236.4 ± 248.2	1309.5 ± 201.1 [f]	73.1 ± 47.1
CHO	843.3 ± 46.0	834.7 ± 63.4	-8.6 ± 17.4	766.4 ± 54.9	903.6 ± 73.2	137.2 ± 18.3
PLA	872.3 ± 81.0	886.5 ± 112.0	14.2 ± 31.0	945.3 ± 116.2	864.0 ± 122.2	-81.3 ± 8.0
IL-1B (pg/mL)						
CM	2.87 ± 1.59	3.15 ± 1.41 [a]	0.28 ± 0.18	2.45 ± 1.30	7.38 ± 3.27 [a]	4.93 ± 1.97
CHO	5.46 ± 1.61	9.09 ± 3.10 [a]	3.63 ± 1.49	4.46 ± 1.59	8.55 ± 3.03 [a]	4.09 ± 1.44
PLA	5.27 ± 2.08	6.27 ± 1.63 [a]	1.00 ± 0.45	6.00 ± 2.94	7.28 ± 2.62 [a]	1.28 ± 0.32
IL-6 (pg/mL)						
CM	3.81 ± 2.15	6.76 ± 4.24 [a]	2.95 ± 2.09	3.69 ± 2.49	8.62 ± 3.03 [a]	4.93 ± 0.54
CHO	7.38 ± 5.56	14.29 ± 8.32 [a]	6.91 ± 2.76	6.74 ± 3.57	7.76 ± 2.48 [a]	1.02 ± 1.09
PLA	11.73 ± 5.77	16.57 ± 7.35 [a]	4.84 ± 1.58	14.98 ± 7.92	26.70 ± 10.81 [a e]	11.72 ± 2.89
IL-8 (pg/mL)						
CM	3.45 ± 1.16	6.12 ± 1.83 [a]	2.67 ± 0.67	4.89 ± 1.53	7.97 ± 2.61 [a]	3.08 ± 1.08
CHO	13.43 ± 7.56	19.15 ± 10.21 [a]	5.72 ± 2.65	12.59 ± 5.82	20.21 ± 8.20 [a]	7.62 ± 2.38
PLA	15.67 ± 7.24	21.07 ± 8.79 [a]	5.40 ± 1.55	17.25 ± 7.56	24.85 ± 9.87 [a]	7.60 ± 2.31
IL-10 (pg/mL)						
CM	5.08 ± 3.68	9.26 ± 5.02 [a]	4.18 ± 1.34	5.03 ± 3.77	9.97 ± 4.07 [a]	4.94 ± 0.30
CHO	2.62 ± 1.23	4.85 ± 1.68 [a]	2.23 ± 0.22	2.12 ± 1.07	4.47 ± 2.34 [a]	2.35 ± 1.27
PLA	9.10 ± 7.32	13.25 ± 7.03 [a]	4.15 ± 0.29	9.71 ± 7.89	12.13 ± 7.72 [a]	2.42 ± 0.17
IL-1Ra (pg/mL)						
CM	2.58 ± 1.75	9.30 ± 5.99	6.72 ± 4.24	3.31 ± 2.92	8.60 ± 4.11	5.29 ± 1.19
CHO	0.55 ± 0.29	1.29 ± 0.71	0.74 ± 0.42	0.95 ± 0.71	1.88 ± 1.57	0.93 ± 0.86
PLA	5.82 ± 3.98	8.90 ± 5.90	3.08 ± 1.92	7.34 ± 5.19	10.69 ± 6.67	3.35 ± 1.48
TNFα (pg/mL)						
CM	8.90 ± 3.31	11.04 ± 3.96	2.14 ± 0.65	8.89 ± 3.22	10.35 ± 2.86 [a]	1.46 ± 0.36
CHO	8.68 ± 2.16	9.65 ± 2.53	0.97 ± 0.37	9.03 ± 2.30	9.92 ± 2.45 [a]	0.89 ± 0.15
PLA	11.51 ± 2.89	16.84 ± 6.91	5.33 ± 4.02	10.89 ± 2.74	17.49 ± 5.50 [a]	6.60 ± 2.76

Values are mean ± SE. Significant differences: [a] significant time effect from pre- to post-exercise; [c] CM vs CHO; [e] PLA vs CHO; [f] CM vs CHO and PLA (P<0.05).

Table 6.6. Pre- and post-exercise blood and plasma measures

	Baseline	Midpoint	End	Pre- and Post Exercise			
				TT 2 Pre	TT2 Post	TT 4 Pre	TT 4 Post
WBCs (x10^3/μL)							
CM	5.59 ± 0.36	5.75 ± 0.68	5.36 ± 0.49	6.45 ± 0.43	10.44 ± 0.69 [a]	6.15 ± 0.46	10.22 ± 0.77 [a]
CHO	6.08 ± 0.52	5.77 ± 0.26	6.15 ± 0.42	6.15 ± 0.30	8.77 ± 0.45 [a]	6.63 ± 0.28	9.95 ± 0.68 [a]
PLA	6.14 ± 0.65	5.02 ± 0.54	5.57 ± 0.56	6.32 ± 0.50	10.20 ± 1.38 [a]	5.98 ± 0.34	10.24 ± 0.91 [a]
Lymphocytes (x10^3/μL)							
CM	2.36 ± 0.14	2.30 ± 0.28	2.38 ± 0.24	2.52 ± 0.25	3.96 ± 0.27 [a]	2.48 ± 0.17	4.35 ± 0.35 [a]
CHO	2.71 ± 0.23	2.32 ± 0.15	2.33 ± 0.22	2.39 ± 0.08	3.52 ± 0.26 [a]	2.80 ± 0.22	4.33 ± 0.29 [a]
PLA	2.47 ± 0.31	2.06 ± 0.22	2.25 ± 0.23	2.52 ± 0.20	3.26 ± 0.21 [a]	2.50 ± 0.22	3.70 ± 0.28 [a]
Neutrophils (x10^3/μL)							
CM	2.54 ± 0.32	2.81 ± 0.51	2.33 ± 0.28	3.32 ± 0.28	5.65 ± 0.60 [a]	2.97 ± 0.35	5.05 ± 0.62 [a]
CHO	2.50 ± 0.45	2.72 ± 0.17	2.93 ± 0.50	2.99 ± 0.21	4.10 ± 0.30 [a]	3.02 ± 0.18	4.41 ± 0.51 [a]
PLA	3.08 ± 0.39	2.28 ± 0.31	2.89 ± 0.36	3.12 ± 0.33	6.25 ± 1.49 [a]	2.62 ± 0.26	5.59 ± 0.97 [a]
Monocytes (x10^3/μL)							
CM	0.46 ± 0.05	0.39 ± 0.06	0.48 ± 0.06	0.43 ± 0.04	0.65 ± 0.06 [a]	0.46 ± 0.04	0.60 ± 0.06 [a]
CHO	0.51 ± 0.05	0.41 ± 0.03	0.58 ± 0.05	0.48 ± 0.04	0.70 ± 0.05 [a]	0.51 ± 0.04	0.80 ± 0.09 [a]
PLA	0.43 ± 0.06	0.39 ± 0.05	0.44 ± 0.06	0.47 ± 0.05	0.73 ± 0.09 [a]	0.44 ± 0.05	0.78 ± 0.09 [a]

Values are mean ± SE. Significant differences: [a] significant time effect from pre- to post-exercise (P<0.05).

Table 6.7. Leukocytes and subpopulations

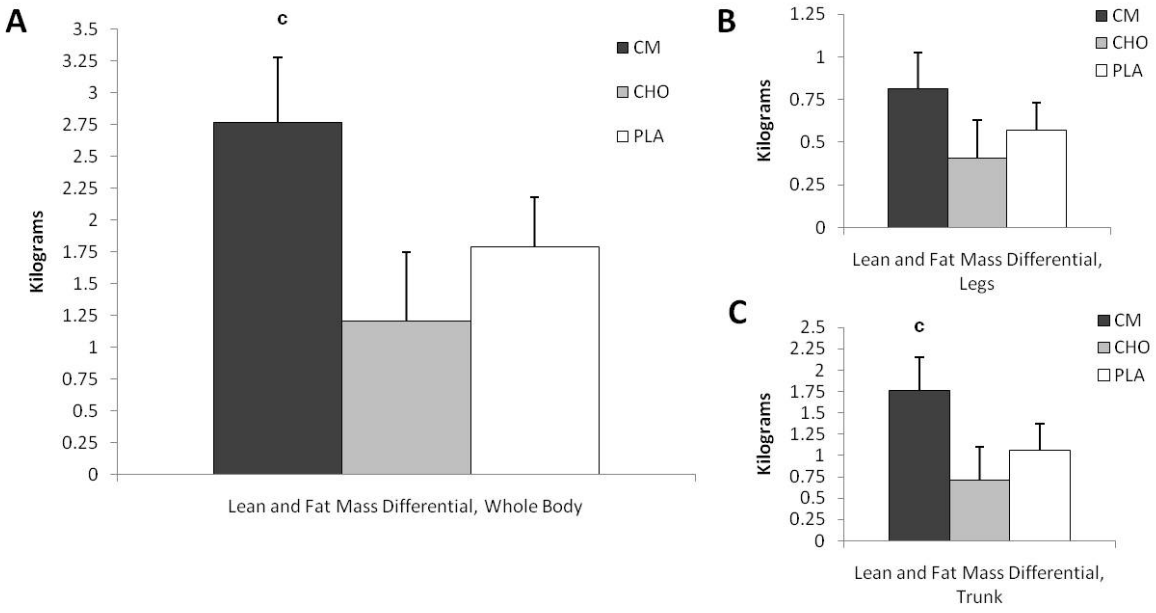

Figure 6.1. Body composition lean and fat mass differentials. A. Whole body differential. Lean mass (kg) gained and fat mass (kg) lost was used to calculate a whole-body differential to quantify overall body mass changes in response to 4.5 wks of cycling exercise training. (LM) – (FM) = differential. Example: (0.900 kg lean mass) - (-0.350 kg fat mass) = 1.250 kg. **B.** Lean and fat mass differential for the legs. **C.** Lean and fat mass differential for the trunk region. Values are mean ± SE. Significant treatment differences: [c] CM vs CHO ($P<0.05$).

Chapter VII: General Discussion

The goal of this series of dissertation studies was to determine the effects of CHO+PRO supplementation on measures of endurance exercise performance, acute recovery and subsequent exercise performance, and adaptations to chronic exercise training at the whole body, systemic and cellular levels. The results of these studies are summarized below.

Several recent investigations have reported significant improvements in endurance exercise performance when a CHO+PRO beverage is ingested during exercise compared to a traditional, isocaloric 6-8% CHO beverage (9, 13-14). However, many athletes and recreational exercisers desire a lower carbohydrate, lower calorie alternative when maintaining or reducing body weight is a goal, in addition to improving fitness and endurance. Therefore, in the first study, we compared a supplement containing a mixture of CHO (dextrose, maltodextrin and fructose) plus a moderate amount of protein to a higher CHO (dextrose) supplement on time to exhaustion and a muscle damage marker (myoglobin) during prolonged intense cycling exercise in trained subjects. We found that the lower-CHO, moderate PRO supplement significantly improved aerobic endurance in an intensity-dependent manner. The significant improvement in time to exhaustion was found when subjects were cycling at or below the ventilatory threshold, but above that threshold, there was no difference between treatments in time to exhaustion. No difference in myoglobin was detected between treatments regardless of ventilatory threshold stratification. The improvement in performance occurred in the CHO+PRO treatment despite the supplement containing 50% less CHO and 30% fewer calories relative to the CHO beverage. The data suggest that the use of multiple CHO sources that utilize different intestinal transporters, along with the addition of protein, improved time to exhaustion when cycling at or below the ventilatory threshold. Thus, we conclude that ingestion of a lower-CHO, moderate-PRO supplement taken during endurance exercise can improve time to exhaustion in an intensity-dependent manner, despite containing less carbohydrate and fewer calories than a traditional CHO supplement.

In addition to the benefits of CHO+PRO supplementation when ingested during endurance exercise, several investigations have demonstrated that post-exercise CHO+PRO supplementation can restore muscle glycogen (2, 17), reduce muscle damage (13), and increase time to exhaustion in a subsequent exercise bout (1, 12-13, 17) compared to CHO alone. Post-exercise CHO+PRO supplementation has also been shown to alter the phosphorylation of signaling proteins related to protein synthesis (6, 11), although the effects when compared to CHO only and placebo (PLA) in an endurance exercise model has not been thoroughly characterized. Muscle protein degradation-specific signaling has also not been well characterized in endurance exercise in response to CHO+PRO supplementation. While most investigations have used commercially-available CHO+PRO supplements, we investigated the effects of a natural food, flavored chocolate milk (CM) as a post-endurance exercise CHO+PRO supplement compared to CHO and PLA on measures of recovery from an intense exercise bout in the second study. We found no difference between CM and CHO in muscle glycogen resynthesis over a 4 h recovery, although both treatments were significantly higher than PLA. However, subsequent time trial performance was significantly faster in the CM treatment, and the activation states of proteins involved in the initiation of mRNA translation for protein synthesis was greater than CHO or PLA. Both the CM and CHO treatments were more effective than PLA in reducing the signaling for protein degradation as assessed by FOXO3A phosphorylation.

Additional important findings with regard to signaling protein activation were that the timing, as well as the nutrient content, of supplementation is critical for optimizing the recovery and adaptive processes. It has previously been shown that the increase in muscle mass is greater (3-5, 8, 15-16), and recovery and subsequent performance is improved (1) when supplementation is provided immediately rather than several hours post-exercise. In the second study, we demonstrated that when either CM or CHO supplementation is provided immediately post-exercise, the phosphorylation of key signaling proteins involved in muscle protein synthesis (e.g., mTOR and rpS6) is greater at 45 min of recovery (R45) than at baseline (R0), whereas in the absence of nutrients

(e.g., placebo), the phosphorylation status did not change. By the end of recovery, the signal in CM was lower than at R45, although still significant from R0. In the CHO treatment, mTOR and rpS6 phosphorylation had returned to the R0 value at the end, and PLA remained unchanged. The phosphorylation status of the muscle protein degradation signaling protein FOXO3A followed the same pattern at R45, with both nutrient-containing treatments showing significantly greater phosphorylation from R0. At the end of recovery, the signal was still elevated in CHO compared to the R0 value. These lowered or non-significant end of recovery phosphorylation signals occurred despite ingestion of another dose of supplement at 2 h into recovery. Thus, these data suggest that if supplementation was delayed rather than ingested immediately post-exercise, the phosphorylation status of these important proteins involved in protein synthesis and degradation would have been diminished, and the opportunity for positive adaptation and optimized adaptation lessened.

The nutrient content of the post-exercise supplementation is also of great importance. The findings from the second study also show that mTOR phosphorylation is greater in CM than CHO or PLA (e.g., exercise only) at R45, and in CM compared to PLA at the end of recovery. For rpS6 the findings are similar except that CHO is non-significantly different from CM or PLA at R45. FOXO3A phosphorylation was greater in both CM and CHO compared to PLA at R45, and at 4 h, the phosphorylation signal was higher in CHO compared to PLA. Previous data from our lab have shown significantly greater phosphorylation of mTOR and rpS6 at 45 min post-exercise when a CHO+PRO supplement was ingested compared to PLA immediately after cycling exercise (6), and others have shown that the combination of CHO and either protein or amino acids can have an additive effect on protein synthesis (7, 10). Thus, it can be concluded that while provision of nutrients in the form of carbohydrate or carbohydrate plus protein has significant advantages in activating key proteins involved in protein synthesis compared to the absence of nutrient intake post-exercise, a CM supplement provides the greatest stimulus for protein synthesis. In terms of reducing degradation signaling, both CM and

CHO were more effective than PLA, but CHO demonstrated a stronger signaling stimulus at the end of recovery.

While we demonstrated that post-exercise CHO+PRO supplementation in the form of CM can improve aspects of acute exercise recovery beyond that of CHO alone, the effects of such supplementation in a model of chronic aerobic exercise training has not been previously investigated. Therefore, in the third study, we compared the effects CM, isocaloric CHO, and PLA on cardiovascular and intramuscular adaptations that occurred in response to a 4.5 wk aerobic exercise training program in healthy, untrained subjects. Thirty-two male and female subjects were randomized into treatment groups, and cycled for 1 h/, 5 d/wk for 4.5 wks at 75-80% of VO_2max. Supplements were ingested immediately and 1 h after each session. We found that while aerobic endurance exercise training alone increases VO_2max, muscle oxidative enzyme activity, and the stimulus for mitochondrial biogenesis, exercise training plus CM supplementation yielded greater improvements in cardiorespiratory fitness (VO_2max). Exercise training plus CHO supplementation did not demonstrate this effect. Thus, we concluded that post-exercise CM supplementation during 4.5 wks of aerobic exercise training improved the magnitude of cardiovascular adaptations more effectively than isocaloric CHO or PLA.

The fourth study demonstrated that post-exercise CM supplementation during 4.5 wks of aerobic training improves body composition more effectively than isocaloric CHO or PLA. While all groups lost fat mass and gained lean mass in response to the exercise training, only in the CM group was this change significant from PLA when expressed as a whole body differential. This is a significant and novel finding, given the relatively short duration of the training program, the lack of a resistance training component, and the lack of a controlled, calorie-restricted dietary intervention.

In the fourth study, we also demonstrated that 4.5 wks of training does not appear to perturb resting immune cell concentrations or markers of inflammation and muscle damage. Resting leukocytes and subpopulations were not significantly affected by the treatments or by the exercise training itself. Resting inflammatory markers also demonstrated no detectable perturbations from normal resting levels or between the

treatment groups. The expected increases in immune cells and most inflammatory markers from pre- to post-exercise were also not significantly affected by treatment. Thus, cycling at a moderately vigorous intensity for 1 h/d, 5d/wk for 4.5 wks does not appear to stress the immune system or increase the inflammatory response in untrained subjects.

Taken together, the collective findings of these studies four suggest that CHO+PRO supplementation extends endurance performance, and that CHO+PRO supplementation in the form of CM improves recovery and increases training adaptations more effectively than CHO or PLA. Key applications of these findings may be important to several populations, such as athletes and recreational exercisers who seek to improve their fitness level and body composition, or those who have endurance and stamina-requiring occupations, such as firefighters, search and rescue personnel and military Special Forces. Clinically relevant applications can extend to older people who exercise regularly and seek to maintain muscle mass and strength, or individuals of any age who must regain muscle mass and strength following an illness or injury.

FUTURE DIRECTIONS

This series of studies has demonstrated many strengths of CHO+PRO supplementation and has addressed some of the potential mechanisms through which this supplementation is beneficial for endurance exercise performance, recovery and training adaptation. However, several areas warrant further investigation.

The most elusive aspect of these investigations is determining the exact mechanism(s) through which CHO+PRO supplementation actually works to improve performance. This could perhaps be elucidated by investigating additional measures of substrate utilization during exercise such as Kreb's cycle intermediates, measuring the gastric emptying rate of the supplements, and using stable isotope methodology to determine the oxidation rates of the different types of carbohydrates in the supplements.

In addition, there are expensive and sophisticated techniques that could address some of the most complex issues of both glycogen and protein synthesis, but these would

have to been done in collaboration with outside facilities. For example, it would be less invasive to address how supplementation affects liver glycogen replenishment through the use of NMR spectroscopy. The use of stable isotope infusions to determine the effects of CHO+PRO supplementation on whole-body protein kinetics and fractional synthetic rates in a training protocol similar to the one employed here would be beneficial as well.

The effects of endurance exercise and nutritional supplementation on the immune system remains open to more investigation. Dividing training groups into intensity categories (e.g., low, moderate, and high intensity training groups) or administering an inhaled rhinovirus challenge would be interesting ways of further characterizing effects of exercise and supplementation on immune response. Characterizing the response of NK cells, and measuring functional properties of phagocytic cells would also better elucidate the effects of exercise and supplementation in periods of training in previously untrained individuals. In addition, methodological modifications such as adding a resting control (no exercise or supplementation) group, providing supplementation during as well as after each exercise bout, and increasing the duration of the training program to ~10-12 wks would possibly allow for significant differences to be found.

Expanding investigations of CHO+PRO supplementation on acute exercise recovery to other populations such as elderly subjects, or using a different exercise mode, such as running or resistance exercise, would broaden our understanding of how exercise and supplementation affects recovery from, and adaptation to, different types of exercise, and make results more generalizable to a wider group of exercisers. Expanding the training model by adding groups including a resistance training only group, and an aerobic exercise plus resistance training group, would lead to greater understanding of the effects of exercise and supplementation on body composition and measures of health. Applying this model to populations such as obese, insulin resistant, or elderly subjects would have important implications for improving our understanding of how exercise and supplementation improves health.

REFERENCES

1. Berardi JM, Noreen EE, and Lemon PW. Recovery from a cycling time trial is enhanced with carbohydrate-protein supplementation vs. isoenergetic carbohydrate supplementation. J Int Soc Sports Nutr 5: 24, 2008.
2. Berardi JM, Price TB, Noreen EE, and Lemon PW. Postexercise muscle glycogen recovery enhanced with a carbohydrate-protein supplement. Med Sci Sports Exerc 38: 1106-1113, 2006.
3. Cribb PJ, and Hayes A. Effects of supplement timing and resistance exercise on skeletal muscle hypertrophy. Med Sci Sports Exerc 38: 1918-1925, 2006.
4. Doi T, Matsuo T, Sugawara M, Matsumoto K, Minehira K, Hamada K, Okamura K, and Suzuki M. New approach for weight reduction by a combination of diet, light resistance exercise and the timing of ingesting a protein supplement. Asia Pac J Clin Nutr 10: 226-232, 2001.
5. Esmarck B, Andersen JL, Olsen S, Richter EA, Mizuno M, and Kjar M. Timing of postexercise protein intake is important for muscle hypertrophy with resistance training in elderly humans. J Physiol 535: 301-311, 2001.
6. Ivy JL, Ding Z, Hwang H, Cialdella-Kam LC, and Morrison PJ. Post exercise carbohydrate-protein supplementation: phosphorylation of muscle proteins involved in glycogen synthesis and protein translation. Amino Acids 35: 89-97, 2008.
7. Levenhagen DK, Carr C, Carlson MG, Maron DJ, Borel MJ, and Flakoll PJ. Postexercise protein intake enhances whole-body and leg protein accretion in humans. Med Sci Sports Exerc 34: 828-837, 2002.
8. Levenhagen DK, Gresham JD, Carlson MG, Maron DJ, Borel MJ, and Flakoll PJ. Postexercise nutrient intake timing in humans is critical to recovery of leg glucose and protein homeostasis. Am J Physiol Endocrinol Metab 280: E982-993, 2001.
9. Martinez-Lagunas V, Ding Z, Bernard JR, Wang B, and Ivy JL. Added Protein Maintains Efficacy of a Low-Carbohydrate Sports Drink. J Strength Cond Res 2009.
10. Miller SL, Tipton KD, Chinkes DL, Wolf SE, and Wolfe RR. Independent and combined effects of amino acids and glucose after resistance exercise. Med Sci Sports Exerc 35: 449-455, 2003.
11. Morrison PJ, Hara D, Ding Z, and Ivy JL. Adding protein to a carbohydrate supplement provided after endurance exercise enhances 4E-BP1 and RPS6 signaling in skeletal muscle. J Appl Physiol 104: 1029-1036, 2008.
12. Niles ES, Lachowetz T, Garfi J, Sullivan W, Smith JC, Leyh BP, and Headley SA. Carbohydrate-Protein Drink Improves Time to Exhaustion after Recovery from Endurance Exercise. JEPonline 4: 45-52, 2001.
13. Saunders M, Kane M, and Todd K. Effects of a Carbohydrate-Protein Beverage on Cycling Endurance and Muscle Damage. Med Sci Sports Exc 36: 1233-1238, 2004.
14. Saunders MJ, Luden ND, and Herrick JE. Consumption of an oral carbohydrate-protein gel improves cycling endurance and prevents postexercise muscle damage. J Strength Cond Res 21: 678-684, 2007.

15. Suzuki K, Totsuka M, Nakaji S, Yamada M, Kudoh S, Liu Q, Sugawara K, Yamaya K, and Sato K. Endurance exercise causes interaction among stress hormones, cytokines, neutrophil dynamics, and muscle damage. J Appl Physiol 87: 1360-1367, 1999.
16. Suzuki M. Glycemic carbohydrates consumed with amino acids or protein right after exercise enhance muscle formation. Nutr Rev 61: S88-94, 2003.
17. Williams MB, Raven PB, Fogt DL, and Ivy JL. Effects of Recovery Beverages on Glycogen Restoration and Endurance Exercise Performance. Journal of Strength and Conditioning Research 17: 12-19, 2003.

Appendix A: Blood Substrate and Hormone Measurement

Blood Collection and Sample Preparation

Glucose

Insulin

Lactate

Free Fatty Acids

Glycerol

Cortisol

Blood Collection and Sample Preparation

Immediately upon collection, each blood sample was anticoagulated with 0.3 ml of EDTA (24mg/ml, pH 7.4). An 0.5 ml aliquot of the anticoagulated blood was transferred to another tube containing 1 ml 10% perchloric acid (PCA). All tubes were centrifuged at 4˚ C for 10 min at 3,000 rpm with a HS-4 rotor in a Sorvall RC6 centrifuge. Plasma and PCA extracts were then aliqoutted to the appropriate number of separate storage tubes and stored at -80˚ C until analyzed.

Plasma Glucose

Plasma glucose was measured using Glucose Color Reagent Kits (Cliniqa/Raichem, San Diego, CA). This method used glucose oxidase (GOD) and a modified Trinder color reaction (Trinder, 1969), catalyzed by peroxidase. GOD oxidized glucose to D-gluconate and formed an equimolar amount of hydrogen peroxide. Hydrogen peroxide oxidatively coupled 4-aminoantipyrine (4-AAP) and p-hydroxybenzene sulfonate (p-HBS) to form a quinoneimine dye in the presence of peroxidase. This dye was intensely red in color, and the intensity of the color in the reaction solution was proportional to the glucose concentration in the sample. Three different concentrations of glucose were used as standards and controls to monitor the performance of assay procedures. All samples were run in duplicate. The coefficient of variation for this assay is shown in the table below. If the difference between duplicate results of a sample was >7% CV, the sample measurement was repeated. According to the manufacture's specifications for this kit, normal fasting ranges were 70-99 mg/dl in healthy, non-diabetic subjects.

Within and between assay variation was performed on three standards containing varying concentrations of glucose. Data (% CV) shown are from two duplicate determinations of each sample in seven separate assays.

Sample No.	Mean mg/dl	Within % CV	Between % CV
1	50	1.9	9.3
2	100	3.7	7.3
3	200	3.8	2.1

Plasma Insulin

Plasma insulin was measured using ImmuChem^{TM} Coated Tube ^{125}I RIA Kit (MP Biomedicals, LLC, Orangeburg, NY) by radioimmunoassay, which was dependent on the binding of an antibody to its antigen. A labeled antigen, Insulin ^{125}I, was added to anti-insulin coated tubes along with the plasma samples or the insulin standards. The labeled antigen and the unlabeled antigen competed to bind with the insulin antibody. The coated tubes were then counted in a gamma counter (1470 Wizard Automatic Gamma Counter, PerkinElmer, Inc., Waltham, MA) calibrated for ^{125}I to measure the concentration of the labeled antigen-antibody complex. Seven insulin standards (0, 5.5, 15, 35, 175, and 310 μIU/mL) were provided in the kit and were used to generate a standard curve. After determining the average counts per sample, the percentage of radioactive insulin bound to the antibody (%B/Bo) was calculated by the following formula:

%B/Bo = (CPM of standard or sample/ CPM of 0 μIU/mL) x 100

The insulin concentrations of the plasma samples were then calculated using the equation of the standard curve. According to the manufacturer's specifications, fasting levels of insulin using the ImmuChem^{TM} Insulin RIA kit generally yielded a range of 4.3-19.9 μIU/mL (mean = 9.6, n = 24). The minimum detectable dose using this kit was specified to be 4.6 μIU/mL. Intra-assay and inter-assay variations are shown in the table below.

		Intra-Assay Variation(m=1)			**Inter-Assay Variation(m=12)**		
Control	n	Mean	SD	CV%	Mean	SD	CV%
Pool-1	12	18.19	1.50	8.25	19.07	1.68	8.81
Pool-2	12	36.49	1.54	4.22	35.89	3.04	5.66
Pool-3	12	91.42	4.93	5.39	8.81	8.47	6.36

m=number of assays n=number of determinations

All samples were run in duplicate, and 2 controls were used as a quality control for the instrument, reagents and technique.

Blood Lactate

Blood lactate was determined from the PCA extract by an enzymatic-spectrophotometric method based on oxidation of lactate to pyruvate by nicotinamide adenine dicnucleotide (NAD^+) according to the protocol of Hohorst (1963). Lactate dehyrdogenase catalyzed the following reaction:

Lactic Acid + NAD^+ $\leftrightarrow$ pyruvic acid + NADH

Lactic acid was converted to pyruvate with the addition of excess NAD^+ and LDH. NADH was measured spectrophotometrically at 340 nm (Hohorst, 1963). To ensure that the reaction went to completion, pyruvate was removed by the addition of hydrazine, which formed a complex with pyruvic acid. All samples were run in duplicate. Normal resting, fasted lactate levels ranged between 0.8 and 1.5 mM.

Plasma Free Fatty Acids

Plasma free fatty acid (FFA) concentration was measured using the colorimetric assay procedure of Duncombe (1964) but modified by using the extraction reagent of Noma et al. (1973) and the copper reagent of Laurell and Tribbling (1967). The principle of the assay was based on the extraction of FFAs from the plasma using a low concentration of methanol with the nonpolar solvents chloroform and heptane. Using this combined solvent, FFAs formed copper salts when added to a copper-TEA (triethanolamine) solution. Then, the copper salts reacted with the sodium salt of diethyldithiocarbamic acid (DDC), forming a colored product. Absorbance at 436 nm is read 10 min after the addition of the DDC to the nonpolar solvent, the copper-TEA solution. Five concentrations of FFAs were used as standards for the formation of a standard curve: 0.125 mM, 0.25 mM, 0.5 mM, 1 mM, and 2 mM. Values were obtained from a standard curve calculated from a linear regression equation ($y = a + bx$) derived from the absorbance of standards. A control sample of known FFA concentration was used as a quality control for the instrument, reagents and technique. Within and between assay variation calculations were performed on four standards containing varying

concentrations of FFAs. The %CV from two duplicate determinations of each sample in 4 separate assays is shown in the table below.

Sample No.	Mean µmol/mL	Within % CV	Between % CV
1	0.25	5.5	5.7
2	0.5	5.6	5.9
3	1	4.9	2.7
4	2	5.1	2.1

If the difference between duplicate results of a sample was >10% CV, the sample measurement was repeated. All samples were read in duplicate. The normal fasting range for FFA concentration is 0.3-0.8 mM.

Plasma Glycerol

Plasma glycerol concentration was measured from the PCA extract according to the protocol of Weiland (1974).The principle of this assay is based upon the phosphorylation of glycerol, followed by its metabolism to dihydroxyacetone phosphate (DHAP), with concomitant production of NADH. Glycerol kinase (GK) converts glycerol and ATP to glycerol-3-phosphate and ADP. Glycerol-3-phosphate, along with NAD+, is then converted into DHAP and NADH, respectively, by glycerol-3-phosphate dehydrogenase (GPDH). The amount of NADH produced is proportional to the amount of glycerol present in the sample and can be read fluorometrically or spectrophotometrically at 340 nm (Varian Cary Eclipse, Varian, Inc., Palo Alto, CA). A standard curve was generated from the concentration values (y axis) vs fluorescence (x axis), and sample concentrations were calculated from the linear regression equation. Values were then multiplied by the dilution factor of 3, and values are expressed in mM. All samples were read in duplicate. Normal values typically are expected to range from 0.01 - 0.3 mM.

Plasma Cortisol

Cortisol levels in plasma were measured using ImmuChem^{TM} Coated Tube ^{125}I RIA Kit (MP Biomedicals, LLC, Orangeburg, NY) by radioimmunoassay, which is dependent on the binding of an antibody to its antigen. A labeled antigen, Cortisol ^{125}I, was added to anti-cortisol coated tubes along with the plasma samples or the cortisol

standards. The labeled antigen and the unlabeled antigen compete to bind with the cortisol antibody. The coated tubes were then counted in a gamma counter (1470 Wizard Automatic Gamma Counter, PerkinElmer, Inc., Waltham, MA) calibrated for ^{125}I to measure the concentration of the labeled antigen-antibody complex. Six standards (0, 1.0, 3.0, 10, 30, and 100 μg/dL) were provided in the kit and were used to generate a standard curve. After determining the average counts per sample, the percentage of radioactive cortisol bound to the antibody (%B/Bo) was calculated by the following formula: %B/Bo = (CPM of standard or sample/ CPM of 0 μg/dL) x 100]

The percent bound was plotted versus the concentration of the cortisol standards, yielding the standard curve. The cortisol concentrations of the plasma samples were then calculated using the equation of the standard curve. The intra-assay CV for this assay was 8.9% for low concentration ranges (4.7 μg/dl), 5.3% of medium concentration ranges (18.1 μg/dl), and 6.1% for high concentration ranges (47.4 μg/dl). Inter-assay CVs were 9.3% for low concentration ranges (4.96 μg/dl), 7.5% for medium concentration ranges (18.7 μg/dl), and 7.6% for high concentration ranges (50.0 μg/dl). According to the manufacturer's specifications, the assay sensitivity was approximately 0.17μg/dl. All samples were run in duplicate, and 2 controls were used as a quality control for the instrument, reagents and technique.

Appendix B: Measurement of Plasma Muscle Damage Markers

Myoglobin

Creatine Phosphokinase

Myoglobin

Myoglobin was determined by solid phase ELISA (BioCheck, Inc., Foster City, CA). The test is based on directing a monoclonal antibody against a distinct antigenic determinant on the myoglobin molecule. Solid phase immobilization is accomplished by binding with mouse monoclonal anti-myoglobin antibody. A goat anti-myoglobin antibody is contained in the antibody-enzyme conjugate solution (horseradish peroxidase). When the test sample reacts with the two antibodies, the myoglobin molecules become sandwiched between the solid phase and enzyme-linked antibodies. After incubating at room temperature for 45 min, the wells were washed to remove any unbound labeled antibodies. Then, the wells incubated in tetramethyl benzidine (TMB) reagent for 20 min, which resulted in the development of a blue color. This color development was stopped with the addition of a stop solution, which changes the color to yellow. Myoglobin concentration was directly proportional to the color intensity of the sample, with the absorbance measured spectrophotometrically at 450 nm. All samples were run in duplicate, and 2 controls were used as a quality control for the instrument, reagents and technique. According to manufacturer's specifications, the lowest detectable concentration of myoglobin by this assay is ~5 ng/ml. Precision (intra-assay and inter-assay) is shown in the tables below:

Intra-assay precision

Sample	**1**	**2**	**3**	**4**	**5**
# replicates	20	20	20	20	20
Mean Myo (ng/ml)	55.6	214.3	294.9	505.9	1,437
Std Dev	2.2	12.9	16.2	26.3	94.0
% CV	3.9%	6.0%	5.5%	5.2%	6.6%

Inter-assay precision

Sample	**1**	**2**	**3**	**4**	**5**
# replicates	35	35	35	35	35
Mean Myo	59.2	244.4	330.5	568.3	1451.7
Std Dev	4.6	12.8	38.9	52.7	104.7
% CV	7.8%	5.2%	11.8%	9.3%	7.2%

Creatine phosphokinase

Creatine phosphokinase (CPK) was determined spectrophotometrically using the Creatine Kinase Reagent Kit (Pointe Scientific, Inc., Canton, MI). CPK catalyzes the reversible phosphorylation of ADP to form ATP from ADP and creatine phosphate. However, CPK activity is determined by the rate of production of NADH from additional reactions that follow the initial CPK-catalyzed reaction, as shown below:

$$\text{ADP} + \text{Creatine Phosphate} \xrightarrow{\textbf{CPK}} \text{Creatine} + \text{ATP}$$

$$\text{ATP} + \text{Glucose} \xrightarrow{\textbf{HK}} \text{ADP} + \text{Glucose-6-Phosphate (G6P)}$$

$$\text{G6P} + \text{NAD}^{+} \xrightarrow{\textbf{G6PDH}} \text{6-Phosphogluconate} + \text{NADH}\ \text{H}^{+}$$

Glucose is phosphorylated by hexokinase to form glucose-6-phosphate (G6P). G6P is then oxidized to 6-phosphogluconate, with concomitant NADH production. The rate of NADH formation is directly proportional to CPK activity when measured spectrophotometrically at 340 nm. According to the manufacturer's specifications, within-run CV is reported to be 0.9%, and run-to-run CV is reported to be 1.0%. Regarding sensitivity, 1 u/L of CPK is expected to yield a ΔAbs/min of 0.00015. Expected resting values for males are up to 160 U/L, and females range up to 130 U/L when the assay is performed at 37^{o} C. All samples were run in duplicate.

Appendix C: Measurement of Plasma Cytokines

Cytokines

Total plasma concentrations of interleukin-1 receptor antagonist (IL-1ra), interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-10 (IL-10), and tumor necrosis factor alpha (TNFα) were determined using Millipore High Sensitivity Multiplex Cytokine Assay kits (Millipore, Billerica, MA) read on a Bio-Plex 200 multiplex suspension array system (Bio-Rad, Hercules, CA). This system uses Luminex xMAP detection technology (Luminex Corp., Austin, TX), in which fluorescently dyed 5.6 μm polystyrene microspheres (or beads) to which antibodies are bound as the solid phase of the assay rather than a coated well used in traditional ELISA. Each bead set is is conjugated with a specific reactant for a single cytokine. A bead set for each cytokine of interest is provided in the kit, and the beads are carefully mixed by sonication and vortexing prior to being added to the sample wells. In order to optimize detection, high sensitivity kits were used, as well as an overnight (17 h) incubation period according to the manufacturer's recommendations.

The assays utilize a sandwich capture format. Antibody against the specific cytokine of interest is covalently coupled to the beads and reacts with a sample containing an unknown quantity of cytokine (or standard with known cytokine quantity). After performing a series of washes to remove any unbound protein, a biotinylated detection antibody specific to a different epitope on the cytokine of interest is introduced, resulting in the formation of a sandwich of antibodies around the specific cytokine. The reaction mixture is then incubated with streptavidin-phycoerythrin (streptavidin-PE) conjugate, which serves as the reporter molecule as it gives a fluorescent signal when passing through a laser as described below. The reporter binds to the detection antibodies, completing the reaction on the bead surface. The beads in each well are drawn up in to the Bio-Plex suspension array system's flow cytometer, where they pass rapidly through a first laser which excites the dyes that mark the bead set specific to each individual cytokine. The beads then pass through a second laser that excites the streptavidin-PE, which is the fluorescent dye on the reporter molecules. This step allows the number of

reactions occurring on the surface of each cytokine-specific bead to be quantified. A high-speed digital signal processor identifies each individual bead and quantifies the cytokine assay results based on fluorescent reporter signals to determine the total amount of each cytokine present in each sample well. All samples were run in duplicate, and 2 controls were used as a quality control for the instrument, reagents and technique. The average %CV for this assay was 10%. The Bio-Plex 200 system was validated prior to running the kits in each study, and the system was calibrated each day before the plates were read.

Appendix D: Muscle Biopsy Procedure

The subject's thigh was cleansed with 10% betadine solution and then 1.6 ml of a local anesthesia (1% Lidocaine Hydrochloride Injection, Elkins-Sinn, Inc., Cherry Hill, NJ) was injected into the subcutaneous region above the vastus lateralis using a 25-guage hypodermic needle. A 5 mm incision was made through the anesthetized skin and fascia with a sterile scalpel, 2.5 inches from the midline of the thigh on the lateral side and 6.0 inches above the patella. Once the bleeding was stopped with direct pressure over the incision, the muscle biopsy was taken using a 3.5 to 5 mm sterile biopsy needle with accompanying suction. The sample size was approximately 50 to 80 mg. Pressure was immediately reapplied to the incision area to stop any bleeding. The biopsy sample was trimmed of adipose and connective tissue and immediately frozen in liquid nitrogen at -80^{o}C for subsequent analysis. Once bleeding had stopped, the incision was closed with two skin closure strips, and a Band-Aid was applied on top of the strips. A pressure pack was affixed firmly over the incision and taped in place with athletic tape and adhesive wrap. The subjects were given verbal and written instructions on how to care for the biopsy site and were required to return for a recheck and redressing of the site 24 hours later.

Appendix E: Homogenization for Glycogen Measurement and Western Blotting (Study 2)

SOLUTIONS:

Homogenization buffer, pH 7.4 (for 200 mL):

0.9532 g of 20 mM HEPES (H-3375)
0.1522 g of 2 mM EGTA (E-4378)
0.418 g of 50 mM NaF (S-7920)
1.492 g of 100 mM KCL (P-9333)
0.0148 g of 0.2 mM EDTA (E-5134)
2.16 g 50 mM Glycerophosphate (G-6521)
0.03 g of 1 mM DTT (D-0632)
200 μL of PMSF stock (0.1 mM)
0.03132 g of 1 mM benzamidine (B-6506)
0.0184 g of 0.5 mM sodium vanadate (S-6508)

PMSF Stock:

17.4 mg PMSF (P-7626) into 1 mL 100% EtOH

PROCEDURE:

1. Weigh muscle samples
2. Homogenize in 9x volume of ice-cold homogenization buffer, pH 7.4, using 3 x 5 sec bursts with a Caframo RZR1 Homogenizer at speed 3-4.
3. Measure exact volume of homogenate using micropipette.
4. Centrifuge homogenates at 1000 rpm for 2 min at 4^{o}C to collect all sample from tube walls.
5. Transfer half of supernatant into separate tube for immediate use in muscle glycogen assay. The remaining half is immediately prepared for use in protein assay.
6. If supernatant samples are not to be used right away, aliquot and store at -80^{o}C until analyzed.

Appendix F: Modified RIPA Buffer Preparation and Homogenization Procedure for PGC-1α Determination

SOLUTIONS:

Modified radioimmunoprecipitation (RIPA) buffer:
Stock Solutions (make in advance and store at 2-8^{o}C until ready for use)

Tris-Hcl, 500 ml (1M, pH 7.4). Sigma T-6066
Add 60.55g Tris base to 400 ml deionized water.
Stir the solution until all solid are dissolved.
Using HCl, adjust pH to 7.4.
Adjust volume of the solution to 500 ml using a graduate cylinder.
Store at 2-8°C.

NaCl, 100 ml (5M, pH 7.4). Fisher S271-3
Add 29.22g NaCl to 60ml deionized water.
Adjust pH to 7.4. Bring volume to 100 ml.
Store at 2-8°C.

Na-deoxycholate, 10 ml (10%). Sigma D5670
Add 1g Na-deoxycholate to 10 ml deionized water.
Store at 2-8°C. Protect stock solution from light.
(Solution will gel after refrigeration; vortex well before use.)

EDTA, 100ml (100mM, pH 7.4). Sigma ED2SS
Add 3.722g EDTA to 80 ml deionized water.
Adjust pH to 7.4.
Bring solution to 100 ml using deionized water.
Store at 2-8°C.

Protease and phosphatase inhibitor stocks:

NaF, 10ml (200mM). Sigma S7920
84 mg NaF to 8 ml deionized water.
Bring solution to 10 ml. Store at RT.

Leupeptin (Sigma L2884) & Aprotinin (Sigma A1153)
1mg/ml stock in deionized water.
Aliquot and store at -20°C.

Pepstatin (Sigma P5318)
1mg/ml stock in HPLC-grade methanol.
Aliquot and store at -20°C.

Sodium Orthovanadate 10 ml (200 mM, pH 10) Fisher S454-50
Activation:
Prepare a 200 mM solution of sodium orthovanadate.
Adjust the pH to 10.0 using either 1 N NaOH or 1 N HCl.
At pH 10.0 the solution will be yellow. Boil the solution until it turns colorless (approx. 3 minutes). Cool to room temperature.
Readjust the pH to 10.0 and repeat the boiling and cooling steps until the solution remains colorless and the pH stabilizes at 10.0.
Aliquot and store at -20°C.

Phenylmethansulfonyl Fluoride 10ml (PMSF, 200mM) Sigma P7626
Add 348 mg PMSF to 10ml isopropanol. Store at RT.
(PMSF is very unstable in aqueous solution and has a half-life of ~30 min. Add immediately before use.)

Modified RIPA buffer preparation:

Chemical	Stock	Final	30 ml
Tris-Hcl, pH 7.4	1M	50mM	600 µl
NaCl, pH 7.4	5M	150mM	90 µl
*Igepal CA-630	10%	1%	300 µl
Na-deoxycholate	10%	1%	300 µl
EDTA pH 7.4	100mM	1mM	300 µl
Na_3VO_4 pH 10	200mM	1mM	150 µl
Aprotinin	1mg/ml	1mg/L	30 µl
Leupeptin	1mg/ml	1mg/L	30 µl
Pepstatin	1mg/ml	1mg/L	30 µl
NaF	200mM	1mM	150 µl
Water			28.2 ml
PMSF	200mM	1mM	150 µl

*Igepal CA-630 (Sigma I8896) is used in place of NP-40.

PROCEDURE:

1. Prepare modified RIPA buffer and add inhibitors to the buffer stock.
2. Weigh muscle samples.
3. Homogenize in 9x volume of ice-cold modified RIPA homogenization buffer using 4 x 5 sec bursts with a Caframo RZR1 Homogenizer at speed 3-4.
4. Freeze at -80°C for 30 min, and thaw over ice (30 min). Vortex well.
5. Repeat freeze-thaw-vortex steps two more times.
6. Sonicate thawed homogenates on ice for 10 sec.
7. Centrifuge homogenates at 5000xg for 20 min at 4°C.
8. Aliquot supernatant and store at -80°C until analyzed.

Appendix G: Muscle Glycogen Measurement

SOLUTIONS:

1 N KOH (for 500 mL):
28.01 g KOH (Fisher P250) into 500 mL ddH_2O

1 N NaOH (for 200 mL):
8 g NaOH (Fisher S318-3) into 200 mL ddH_2O

50% glacial acetic acid (for 200 mL):
100 mL glacial acetic acid (Sigma A9967)
100 mL ddH_2O

10 mg/ml amyloglucosidase in sodium acetate (for 40 mL):
40 mg amyloglucosidase (Sigma 10115)
4 mL 0.3 M sodium acetate, pH 4.8 (Sigma S-8750)

PROCEDURE:

1. Homogenize muscle tissue samples as described in Appendix E.
2. Add 1 ml of ice-cold 1N KOH into each homogenate tube specified for use in the glycogen assay.
3. Incubate the homogenate + 1 ml of 1N KOH at 60^{o}C for 20 min, vortexing vigorously every 5 min.
4. Transfer 100 µl aliquot of the KOH-digested homogenate to 250 µl of 0.3 M sodium acetate, pH 4.8, and vortex.
5. Add 10µl of 50% of glacial acetate acid to each of the tubes.
6. Add 250 µl of 0.3 M sodium acetate, pH 4.8, containing 10 mg/ml amyloglucosidase to each tube and vortex.
7. Seal tubes with parafilm and incubate overnight at room temperature.
8. The following day, add 25 µl of 1 N NaOH to each sample tube and vortex to terminate reaction.
9. Set up 12 x 75 mm test tubes for Raichem Trinder assay for determination of liberated glucose.
10. Prepare Raichem color reagent according to product insert and pipet 1.5 mL of reagent into each tube, including two standards and samples in duplicate and a blank.
11. Pipet duplicate 100 µl aliquots into test tubes for the Trinder reaction to begin. Use 2 duplicate glucose standards (4.505 mg and 9.01 mg glucose/dL dH_2O) to run in parallel with the muscle samples.
12. Incubate at 37 ^{o}C for 15 min in a skaing bath set to speed of 50.
13. Read the absorbance at 500 nm, setting the instrument to 0 by blanking with the prepared blank.

CALCULATIONS:

1. (Ab sample / Ab standard) × (standard conc. = 4.505 mg/dl) = mg/dl
2. Dilution factors
   DF1: (1000 µl + half volume in µl of muscle homogenate)/100µl = µl
   DF2: [(0.25 ml + 0.01 ml + 0.25 ml + 0.025 ml) + 0.1 ml]/0.1 ml = 6.35
   DF3: volume in cuvette, same in standard = 1
3. Final Calculation:
   [(mg/dl) × (0.1 ml/100 ml) × DF1 × DF2 × DF3]/half muscle weight (g) = mg glycogen/g muscle
   {[(mg glycogen/g muscle)/1000]/180.2}*10^6 = µmol glycogen/g muscle

Appendix H: Determination of Muscle Protein Concentration

(Modified Lowry Assay)

SOLUTIONS:

2% Sodium carbonate
20 g Sodium carbonate into 1 L 0.1 N NaOH

0.1 N NaOH
4 g NaOH (Fisher S-318-3) into 1000 mL ddH_2O

1% Cupric sulfate
2 g cupric sulfate (Fisher C-489) in 200 mL ddH_2O

2% Sodium potassium tartrate
4 g sodium potassium tartrate (Fisher S-387) in 200 mL ddH_2O

PROCEDURES:

1. Thaw 1 aliquot of 5 mg/mL BSA stock on ice.
2. Using the half of total homogenized muscle sample as described in Appendix E, centrifuge at 14,000 G for 10 min.
3. For each sample, transfer 7 µl of supernatant to labeled microcentrifuge tube containing 273 µl ddH_2O and vortex.
4. Aliquot the remaining supernatant into 4 0.5 mL microcentrifuge tubes per sample. (Each aliquot will contain was ~30 µl of sample supernatant.) Freeze in liquid nitrogen immediately and quickly store in -80^{O}C freezer. (Freeze pellet separately at -80^{O}.)
5. Perform serial dilution:

	ddH_2O (mL)	Std protein (mL)	[protein] (mg/mL)
Blank	1.0	0	0
A	1.8	0.2 of BSA Stock	.5
B	0.2	0.8 of A	.4
C	0.5	0.5 of B	.2
D	0.5	0.5 of C	.1
E	0.5	0.5 of D	.05
F	0.5	0.5 of E	.025

6. Dilute samples 1:30 with ddH2O (10 µl of sample + 290 µl ddH_2O) or 1:40 with ddH2O (7 µl of sample + 273 µl ddH_2O) and keep on ice.
7. Make Solution A (1 mL per tube needed) in a 125 mL Erlenmeyer flask:

96 mL	2% Sodium carbonate	Stir with stir bar
2 mL	2% Sodium potassium tartrate	Gently layer
2 mL	1% Cupric sulfate	Gently layer

8. Add 0.1 mL of each standard or sample to each corresponding tube and duplicates.
9. Add 1.0 mL of Solution A to every tube except blank. Vortex each tube after Solution A is added to all.
10. Incubate at room temperature for 10 min.
11. Dilute Folin & Ciocalteu's Phenol Reagent (Sigma F-9252) 1:2 with ddH_2O. Stir briefly.
12. After 10 min incubation, pipet 0.1 mL of phenol solution directly into the center of each tube (standards & samples) while vortexing each tube, and without touching sides of tube.
13. Incubate 30 min at room temperature.
14. Turn on spectrophotometer and set to 750 nm.
15. After incubation, and after blanking the spectrophotometer to the prepared blank, read absorbance of all standards and samples in duplicate at 750 nm.
16. Generate standard curve from absorbances of the 6 standards. Using the equation from the standard curve, to calculate the protein amount in the sample (average of each sample and its duplicate). Multiply the amount by the dilution factor (30 or 40) for the final protein concentration in each sample.

Appendix I: SDS-PAGE Procedure

SOLUTIONS:

The following directions are for making stock solutions for the SDS-PAGE procedure:

2x Sample buffer (for 50 mL):
5 mL of 1.25M Tris, pH 6.8
26.2 mL ddH_2O
10 mL glycerol
5 mL 20% SDS (Sigma L-3771)
2.25 mL of 2-mercaptoethanol (Sigma M-6250) (add under fume hood)
1.6 mL of 0.25% bromophenol blue solution

0.25% Bromophenol blue (for 5 mL):
0.0125 g bromophenol blue (Sigma B-5525) in 5 mL ddH_2O

Acrylamide mix (30% mixed acrylamide and 1% bisacrylamide) (for 200 mL):
58 g acrylamide (Sigma A8887)
2 g bisacrylamide (Sigma M7279)
Bring final volume to 200 mL with ddH_2O
Filter through Whatman No. 1 filter paper. Store at 4^oC in a dark bottle.

1.5 M Tris, pH 8.8 (for 500 mL):
90.82 g Trisbase (Sigma T-6066) into 400 mL ddH_2O.
Adjust to 8.8 pH with concentrated HCl.
Bring final volume to 500 mL with ddH_2O and recheck pH.
Store at room temperature.

1.0 M Tris, pH 6.8 (for 500 mL):
60.57 g Trisbase (Sigma T-6066) into 400 mL ddH_2O.
Adjust to 6.8 pH with concentrated HCl.
Bring final volume to 500 mL with ddH_2O and recheck pH.
Store at room temperature.

20% Sodium dodecyl sulfate (SDS) (for 100 mL):
20 g molecular biology-grade SDS (Sigma L-3771) into 100 mL ddH_2O.
Filter through Whatman No. 1 filter paper. Store at room temperature.

10% Ammonium persulfate (APS) (for 1 mL):
100 mg of APS (Sigma A-3678) into 900 μL ddH_2O.
Prepare fresh daily.

TEMED (N,N,N',N'-Tetramethylethylenediamine)
Sigma T9281. Store at 4^oC.

10x Running buffer (for 1 L):
800 mL ddH_2O
30.28 g Trisbase (Sigma T-6066)
144.2 g Glycine (Sigma G-8898)
Bring to 1000 mL ddH_2O

1x Running buffer (for 1 L):
100 mL 10x Running buffer
5 mL of 20% SDS (Sigma L-3771)
900 mL ddH_2O

Resolving Gel (10%)

	For 2 gels (mL)	For 4 gels (mL)
ddH_2O	4.0	7.9
Acrylamide mix	3.3	6.7
1.5 M Tris, pH 8.8	2.5	5.0
20% SDS	0.1	0.2
10% APS	0.1	0.2
TEMED	0.004	0.008

Stacking Gel

	For 2 gels (mL)	For 4 gels (mL)
ddH_2O	3.4	6.8
Acrylamide mix	0.83	1.7
1.5 M Tris, pH 8.8	0.63	1.25
20% SDS	0.05	0.1
10% APS	0.05	0.1
TEMED	0.005	0.01

PROCEDURE:

1. Assemble gel apparatus in casting stands according to manufacturer's instructions.
2. Prepare resolving gel solution for the number of gels to be cast.
3. Gently swirl solution. Use a transfer pipette to fill the caster ~3/4 full. Overlay resolving gel with 200 μL butanol.
4. Allow ~45 min for gel to polymerize.
5. Pour butanol off of the resolving gel and rinse well with ddH_2O. Dry between casting plates as much as possible with a KimWipe.
6. Prepare stacking gel solution for the number of gels to be cast.
7. Gently swirl solution. Use transfer pipette to completely fill rest of the caster to the top with the stacking gel solution. Carefully insert 15-well comb, avoiding any bubble formation.

8. Allow ~45 min for polymerization.
9. During polymerization, prepare samples with sample buffer. Thaw and vortex samples. Dilute the samples 1:2 with 2x sample buffer in 0.5 mL microcentrifuge tubes (e.g., 10.2 μl sample + 10.2 μl sample buffer).
10. Vortex samples + buffer after all samples are prepared. Place tubes in a tube holder, and allow to float in boiling water (~99^{o}C) for 6 min.
11. Transfer tubes into microcentrifuge and spin for ~5 seconds to collect all sample + buffer solution into the bottom of the microcentrifuge tube.
12. Prepare 1x Running buffer.
13. After gels have polymerized, carefully remove the 15-well combs and assemble the gel apparatus and electrophoresis chamber according to the manufacturer's instructions. Fill inner chamber with 1x Running buffer and the outer chamber to ~1/3 full with 1x Running buffer.
14. Load 60 μg muscle homogenate (~10 – 20 μL sample + buffer) and rat muscle standard into wells. Load molecular weight marker in 2-3 wells per gel.
15. Electrophorse at 200 V for 1:34 for gel used for p-mTOR, p-FOXO3A and α-tubulin, 45 min for the gel used for ubiquitination, p-rpS6, p-eIF2B and α-tubulin, and 75 min for PGC-1α.

Appendix J: Western Blotting Procedures

SOLUTIONS:

The following directions are for making stock solutions for the procedure:

Anode I (for 1 L):
800 mL ddH2O
36.33 g Trisbase (Sigma T-6066)
0.5 g SDS (Sigma L-3771)
100 mL Methanol (Fisher A-411-4)
0.78 mL 2-mercaptoethanol (Sigma M-6250) (add under fume hood)
Bring volume to 1000mL with ddH2O

Anode II (for 1 L):
800mL ddH2O
3.025 g Trisbase (Sigma T-6066)
0.5 g SDS (Sigma L-3771)
100 mL Methanol (Fisher A-411-4)
0.78 mL 2-mercaptoethanol (Sigma M-6250) (add under fume hood)
Bring volume to 1000mL with ddH2O

Cathode (for 1 L):
800mL ddH2O
3.025 g Trisbase (Sigma T-6066)
0.5 g SDS (Sigma L-3771)
5.248 g 6-Aminocaproic acid (Sigma A-2504)
100 mL Methanol (Fisher A-411-4)
0.78 mL 2-mercaptoethanol (Sigma M-6250) (add under fume hood)
Bring volume to 1000mL with ddH2O

PROCEDURE:

1. During the last few minutes of the SDS-PAGE procedure, pre-wet the PVDF membranes in methanol, and then soak in Anode I solution for 10 minutes. (Use one PVDF membrane per gel, cut to ~6 cm x 9 cm each. Mark the left edge to identify each membrane to correspond to each gel.)
2. When the gel electrophoresis is complete, remove the resolving gel from the casting plates and soak in Anode I solution for at least 5 min. Make a small cut on the top corner of gel above lane 1 for identification purposes.
3. Briefly soak 2 sheets of filter paper (one think and one thin) in Anode II solution. Place the thin sheet on the Bio-Rad Trans-Blot Semi-Dry electrode. Place the thick sheet directly on top of the thin sheet. Using a glass test tube, press and roll the tube across the sheets to eliminate any bubbles and assure even contact between the two sheets.

4. Remove the PVDF membrane from the Anode I solution and place on top of the filter papers. Remove air bubbles carefully with a glass test tube, wet with Anode I solution.
5. Carefully remove the gel from the Anode I solution and place on top of the PVDF membrane. Remove air bubbles very carefully with a glass test tube, wet with Anode I solution. Take extra care to assure that the gel is straight on the membrane and does not tear.
6. Briefly soak 2 sheets of filter paper (one think and one thin) in Cathode solution. Place the thick sheet on top of the gel and carefully smooth the sheet out with a glass test tube. Place the thin sheet directly on top of the thick sheet. Using a glass test tube, press and roll the tube across the sheets to eliminate any bubbles.
7. Repeat the above process for additional gels. Place the top electrode plate atop the stacks, and place the transfer unit cover securely on the unit.
8. Transfer the proteins from the gel to the PVDF membrane at 25 V for 18 min for the gels and membranes used for p-mTOR and p-FOXO3A, and PGC-1α, and at 25 V for 15 min for gels and membranes used for ubiquitination, p-eIF2B, and p-rpS6.
9. Following the transfer, place the PVDF membrane in ddH_2O and refrigerate at 4^oC until ready to perform immunoblotting procedures.

Appendix K: Immunoblotting Procedures

SOLUTIONS:
The following directions are for making stock solutions:

10x Tris-buffered saline (TBS; pH 7.5) (for 1 L):
800 mL ddH_2O
60.05 g Trisbase (Sigma T-6066)
87.6 g NaCl (Fisher S271-3)
Bring to pH 7.5 with concentrated HCl.
Bring to 1000 mL ddH_2O

1x Tris-buffered saline + 0.06% Tween-20 (1 xTTBS) (for 1 L):
100 mL of 10x Tris-buffered saline (TBS; pH 7.5)
900 mL ddH_2O
0.6 mL Tween-20 (Sigma P-1379)

1x Tris-buffered saline + 0.1% Tween-20 (for 1 L - for PGC-1α)
100 mL of 10x Tris-buffered saline (TBS; pH 7.5)
900 mL ddH_2O
1.0 mL Tween-20 (Sigma P-1379)

Blocking solution: 6% Non-fat dry milk (NFDM) and 1x TTBS (for 100 mL, enough for 2 membranes) (for p-mTOR, p-FOXO3A, p-rpS6 and α-tubulin membranes)
100 mL of 1x TTBS
6 g NFDM

Blocking solution: 5% BSA and 1x TTBS (for 100 mL, enough for 2 membranes)
(for p-eIF2B membranes)
100 mL of 1x TTBS
5 g BSA **(**ICN 105033)
Stir gently on ice for ~1 h until dissolved.

Blocking solution: 10% NFDM and 1x TBS + 0.1% Tween 20 (for 100 mL, enough for 2 membranes) (for PGC-1α membranes)
100 mL of 1x TBS + 0.1% Tween 20
10 g NFDM

Primary Antibody:

	Dilution ratio	Dilution solution	Company and Catalog #
p-mTOR	1:800	2% NFDM-TTBS	Cell Signaling, 2971
p-FOXO3A	1:500	5% BSA-TTBS	Cell Signaling, 9465
p-rpS6	1:1000	2% NFDM-TTBS	Cell Signaling, 2211
p-eIF2Bε	1:1000	1% BSA-TTBS	EMD/Calbiochem, PS1017
Ubiquitin	1:500	2% NFDM-TTBS	Santa Cruz, 9133
PGC-1α	1:1000	3% NFDM-TTBS*	EMD/Calbiochem, 516557
α-tubulin	1:900	2% NFDM-TTBS	Cell Signaling, 2144

Secondary Antibody:

Protein of Interest	Dilution ratio	Dilution solution	Secondary Antibody
p-mTOR	1:800	2% NFDM-TTBS	Goat Anti-rabbit IgG (Cell Signaling 7074)
p-FOXO3A	1:1500	5% NFDM-TTBS	
p-rpS6	1:1500	2% NFDM-TTBS	
p-eIF2Bε	1:3000	1% BSA-TTBS	
Ubiquitin	1:1500	2% NFDM-TTBS	
PGC-1α	1:7500	3% NFDM-TTBS*	
α-tubulin	1:1000	2% NFDM-TTBS	

Stripping buffer, pH 6.7 (for 1 L):
850 mL ddH2O
20g of 2% sodium dodecyl sulphate (SDS) (Sigma L-3771)
7.5 g Trisbase (Sigma T-6066)
Stir, then add 7 mL of 100 mM 2-mercaptoethanol (perform this step under fume hood)
Measure pH
Bring volume to 1000 mL with ddH2O; measure pH again.

PROCEDURE:

1. Block PVDF membranes in appropriate blocking solution (above) for 1 h at room temperature with gentle shaking. (For PGC-1α, block for 2 h)
2. Perform 3 x 5 min washes in 30 mL TTBS
3. Cut PVDF membranes in sections according to molecular weight markers to probe individually for proteins of interest.
4. Incubate each PVDF membrane/membrane section in 10 mL of the respective primary antibody solutions overnight on a rocking platform at low speed (1.5) in 4^{o}C refrigerator.
5. Perform 3 x 5 min washes for each membrane section in 30 mL TTBS.
6. Incubate each PVDF membrane/membrane section in 10 mL of the respective secondary antibody solutions for 100 min (p-mTOR, p-FOXO3A, p-eIF2Bε,

PGC-1α and α-tubulin) or 120 min (ubiquitination and p-rpS6) with gentle shaking at room temperature.

7. Perform 3 x 5 min washes for each membrane section in 30 mL TTBS.
8. Visualize bands using ECL detection (ECL Western Lighting Plus kit, Perkin Elmer) with a Bio-Rad ChemiDoc. Analyze bands (intensity x mm^2) using Quantity One 1.0 1-D Analysis software.

Exposure times:

Protein of Interest	Exposure (sec)
p-mTOR	180
p-FOXO3A	180
p-rpS6	240
p-eIF2Bε	360
Ubiquitin	360
PGC-1α	240
α-tubulin	180

9. Store membranes in ddH_2O in 4^oC.
10. If stripping to reprobe, perform 2 x 5 min washes in 30 mL TTBS.
11. Wash membrane in 30 mL stripping buffer for 1 h in 60^oC shaking water bath at 65 rpm.
12. Perform 3 x 5 min washes for each stripped membrane section in 30 mL TTBS.
13. Start procedures with blocking step and follow procedures forward.

Appendix L: Measurements of Muscle Enzyme Activity

Succinate Dehydrogenase Activity

Citrate Synthase Activity

Succinate Dehydrogenase

SOLUTIONS:

Homogenization buffer (for 500 mL):
35 μL 2-mercaptoethanol, 10mM (Sigma M6250)
25 mg BSA (0.05%) (ICN 105033)
500 mL potassium phosphate buffer, 0.17 M, pH 7.4

0.17 M phosphate buffer, pH 7.4 (for 500 mL):
11.6 potassium phosphate (Sigma P-0662)
500 mL ddH_2O

Incubation medium (for 1 mL):
160 mg sodium succinate (Sigma 52378)
2.6 mg potassium ferricyanide (Sigma 702587)
1 mL 0.17 M phosphate buffer, pH 7.4.

Fluorometric reaction medium (25 mL):
25 mL hydrazine-HCl buffer, 0.2 M, pH 9.2
90 μL 0.1 M NAD, 0.36 mM

Hydrazine-HCl buffer, 0.2 M, pH 9.2 (for 500 mL):
4.9 mL hydrazine hydrate (Sigma H-9507) into 495.1 mL ddH_2O
Bring to pH 9.2 with HCl

NAD, 0.1 M (for 3 ml):
198 mg NAD (Sigma N-7004) into 3 mL ddH_2O

Enzyme mix stock with 10 μg/mL fumarase and 200 μg/mL MDH (for 5 mL):
4.528 mL of 0.17 M phosphate buffer, pH 7.4
454 μL MDH (2.2 mg/mL) (Sigma F-1757)
17.2 μL fumarase (2.9 mg/mL) (Sigma M-9004)

NADH standard, 1 mM (for 10 mL):
7.09 mg NADH (Sigma N-4505) into 10 mL ddH_2O

Deproteinizing and neutralizing reagents:
3 M PCA (for 100 mL):
[(3 x 100.46 MW)/10]/0.70 = 43 mL 70% PCA (Fisher A229) into 57 mL ddH_2O

3 M KOH (for 100 mL):
(3 x 56.11 MW)/10) = 16.8 g KOH (Fisher P250) into ~100 mL ddH_2O

PROCEDURE:

1. Homogenize ~20 mg of frozen tissue in the homogenizing buffer. Dilute 1:70.
2. For each homogenate sample, prepare three 12 x 75 glass culture tubes on ice. Pipet 0.1 mL homogenate into each tube in duplicate (third tube is for blank).
3. Place incubation medium into a 37^{o}C water bath, followed by the sample tubes. Allow about 1 min to reach proper temperature. (Keep blanks on ice.)
4. Add 0.1 mL incubation medium to each sample tube, mix gently, and incubate for exactly 5 min.
5. After the 5 min incubation, stop the reaction by adding 0.1 mL PCA into each tube. Mix and return the tube to the ice bath.
6. Pipet 0.1 mL PCA into blank tubes, followed by adding 0.1 mL incubation medium to each blank.
7. On ice, add 0.1 mL KOH to each tube including blanks. Total volume should be 0.4 mL.
8. Centrifuge all tubes briefly to pellet the potassium perchlorate precipitate and return then to the ice bath.
9. Pipet 1 mL of hydrazine/NAD cocktail into 10 x 75 glass culture tubes.
10. Pipet 50 μL of each sample and blank into a corresponding cocktail tube. Make a tube of 50 μL thawed NADH to use as standard.
11. Add 1.9 mL ddH_2O to each tube.
12. On fluorescent spectrophotometer, read and record initial fluorescence of each tube.
13. Add 25 μL aliquot of enzyme mix to each tube and mix. Let stand at room temperature for 1 hour.
14. Read and record final fluorescence of all tubes. Subtract initial fluorescence from final fluorescence value, as well as the change in the blank fluorescence from the fluorescence change of each sample.

CALCULATIONS:

Calculate the results by the following formula:

(dF_{samp}/dF_{std}) x (mM[std] x mLstd) x $dilution_{samp}$ + grams of tissue + $time_{min}$ = μmol/min/g

Citrate Synthase

SOLUTIONS:

Homogenizing buffer, pH 7.4 (for 500 mL):
6.525 g 0.175 M KCl (Fischer P217)
0.37225 g 2 mM EDTA (sigma E-5134)
500 mL ddH_2O
Bring to pH 7.4 with HCl

Tris buffer, 100 mM, pH 8.3 (for 500 mL):
6.055 g Trizma base (Sigma T-6066) into 500 mL ddH_2O
Bring to pH 8.3 with HCl

DTNB, 1 mM (for 10 mL):
4 mg DTNB (Sigma D-8130) in 10 mL of 100 mM Tris buffer, pH 8.3

Oxalacetate, 10 mM (for 10 mL):
Dissolve 13.2 mg oxalacetate (Sigma O-4126) in 10 mL of 100 mM Tris buffer, pH 8.3

Acetyl Co-A, 3 mM (for 1 mL):
10 mg Coenzyme-A (Sigma C-3144) in 0.9 mL ddH_2O
0.1 mL 1 M $KHCO_3$
0.013 mL acetic anhydride (Fischer A-10)

$KHCO_3$,1 M (for 100 mL):
10.012 mg $KHCO_3$ (Sigma P-184) in 100 mL ddH_2O

0.4% Triton 100 in 0.1 M Tris buffer, pH 8.3 (for 1 mL):
0.8 mL Triton 100 stock solution (Sigma T8787) in 200 mL of 100 mM Tris buffer, pH 8.3

Reagent Cocktail: (Prepare each assay tube individually in the cuvette that will go into the spectrophotometer according to protocol below)
770 μL Tris buffer
30 μL acetyl-CoA
100 μL DTNB (light sensitive; protect from light with foil wrap)
40 μL homogenate (or blank reagent)

PROCEDURE:

1. Homogenize ~20 mg frozen tissue in 1:30 dilution and centrifuge 5 min. Pipet supernatant for 100 μL aliquots. Freeze overnight at -20°C.
2. Next day: Set spectrophotometer for enzyme kinetic assay at 412 nm and 37°C.

3. Thaw the 100 μL homogenates on ice before adding 900 μL 0.4% Triton 100 in 0.1 M Tris buffer. Incubate at room temperature for 20 min.
4. Place empty cuvettes into the spectrophotometer to keep them at 37^{o}C.
5. Add the reagent cocktail reagents (above) to each one, pipet-mixing each time.
6. Blank the spectrophotometer.
7. Measure absorbance for ~90 sec to see if the reagent cocktail is stable.
8. Quickly add 60 μL oxalacetate to each cuvette, pipet-mixing vigorously. Begin reading absorbance at 30 sec intervals. Run for a total of 6 minutes and use the values at 240 sec for calculating results.

CALCULATIONS:

Calculate results based on the molar extinction coefficient for DTNB at 412 nm (13,600) and dilutions of the tissue, as follows:

(ΔAbs/min) x)1/13.6) x dil. of homogenate x dil of tissue in assay vol = μmol/min/g

Appendix M: Substrate Oxidation

Indirect Calorimetry

Fat and Carbohydrate Oxidation Rate Calculations

Fat and Carbohydrate Energy Expenditure Rate Calculations

Indirect Calorimetry

Subjects breathed through a Hans Rudolph valve, with expired gases directed to a mixing chamber for analysis of oxygen (O_2) and carbon dioxide (CO_2) using a ParvoMedics TrueOne2400 metabolic system (ParvoMedics, Sandy, UT). Ventilation, O_2 consumption (VO_2), CO_2 production (VCO_2), and respiratory exchange ratio (RER) was calculated and recorded by the system every 15 s.

Fat and Carbohydrate Oxidation Rate Calculations

Fat and carbohydrate oxidation rates were calculated from the VCO_2 and VO_2 values using the equations of Frayn (1983):

$$\text{CHO oxidation } (g \cdot min^{-1}) = [(4.55 \times VCO_2) - (3.21 \times VO_2) - (2.87n)]$$

$$\text{Fat oxidation } (g \cdot min^{-1}) = [(1.67 \times VO_2) - (1.67 \times VCO_2) - (1.92n)]$$

Where VO_2 and VCO_2 are expressed in $L \cdot min^{-1}$, and n is the nitrogen excretion rate, based on the average of 135 μg/kg/min (Romijn et al, 2000) and estimated for all subjects to be 0.002 μg/kg/min.

Energy Expenditure Rate Calculations

Average CHO and fat energy expenditure rates (EE, $kcal \cdot min^{-1}$) were calculated from the above CHO and fat oxidation rates using the Atwater general conversion factors for CHO and fat (1909):

$$\text{CHO EE } (kcal \cdot min^{-1}) = [(4.55 \times VCO_2) - (3.21 \times VO_2) - (2.87n)] \times 4.2$$

$$\text{Fat EE } (kcal \cdot min^{-1}) = [(1.67 \times VO_2) - (1.67 \times VCO_2) - (1.92n)] \times 9.1$$

Appendix N: Raw Data

Study 1

Study 2

Study 3

Study 4

Study 1	Subject Characteristics: COMBINED GROUP								
Subject	Gender	Age (yr)	Height (cm)	VO_2max (L/min)	VO_2max (ml/kg/min)	VT (L/min)	HR_{max}	$Watts_{max}$	Weight (kg)
BM	M	33	175	4.540	68.85	3.54	189	339	65.9
CJ	M	20	175	4.490	67.85	3.37	197	335	66.6
CW	M	36	180	4.780	55.70	3.49	171	358	85.8
DF	M	26	171	4.005	61.80	3.00	203	296	64.8
EM	M	28	177	4.380	60.90	3.29	207	326	71.9
EMC	F	27	164	2.890	46.15	2.14	172	207	62.6
JB	F	27	159	3.125	53.25	2.31	196	226	58.7
JC	F	26	163	2.275	44.45	1.73	176	158	51.2
JG	M	26	171	4.700	73.45	3.43	186	352	64
JL	M	27	170	4.490	68.80	3.46	178	335	65.3
KJ	F	35	160	1.860	40.40	1.40	189	125	46
KM	F	32	179	3.310	47.00	2.58	183	241	70.4
LM	F	30	183	3.450	46.80	2.59	169	252	73.7
MP	M	35	187	4.465	64.75	3.48	196	333	68.9
MU	F	23	166	3.405	47.05	2.52	186	248	72.4
AVG		**28.7**	**172.0**	**3.7**	**56.5**	**2.8**	**186.5**	**275.4**	**65.9**
SEM		**1.2**	**2.2**	**0.2**	**2.8**	**0.2**	**3.0**	**19.1**	**2.4**

	Subject Characteristics: ABOVE VT								
Subject	Gender	Age (yr)	Height (cm)	VO_2max (L/min)	VO_2max (ml/kg/min)	VT (L/min)	HR_{max}	$Watts_{max}$	Weight (kg)
CJ	M	20	175	4.490	67.85	3.37	197	335	66.6
CW	M	36	180	4.780	55.70	3.49	171	358	85.8
EM	M	28	177	4.380	60.90	3.29	207	326	71.9
JG	M	26	171	4.700	73.45	3.43	186	352	64.0
JL	M	27	170	4.490	68.80	3.46	178	335	65.3
KJ	F	35	160	1.860	40.40	1.40	189	125	46.0
MU	F	23	166	3.405	47.05	2.52	186	248	72.4
AVG		**27.9**	**171.3**	**4.0**	**59.2**	**3.0**	**187.7**	**297.0**	**67.4**
SEM		**2.2**	**2.6**	**0.4**	**4.6**	**0.3**	**4.5**	**31.8**	**4.5**

	Subject Characteristics: AT OR BELOW VT								
Subject	Gender	Age (yr)	Height (cm)	VO_2max (L/min)	VO_2max (ml/kg/min)	VT (L/min)	HR_{max}	$Watts_{max}$	Weight (kg)
BM	M	33	175	4.540	68.85	3.54	189	339	65.9
DF	M	26	171	4.005	61.80	3.00	203	296	64.8
EMC	F	27	164	2.890	46.15	2.14	172	207	62.6
JB	F	27	159	3.125	53.25	2.31	196	226	58.7
JC	F	26	163	2.275	44.45	1.73	176	158	51.2
KM	F	32	179	3.310	47.00	2.58	183	241	70.4
LM	F	30	183	3.450	46.80	2.59	169	252	73.7
MP	M	35	187	4.465	64.75	3.48	196	333	68.9
AVG		**29.5**	**172.6**	**3.5**	**54.1**	**2.7**	**185.5**	**256.5**	**64.5**
SEM		**1.2**	**3.6**	**0.3**	**3.4**	**0.2**	**4.4**	**22.2**	**2.5**

Study 1

Time to Exhaustion		
Combined Group		
Subject	**CHO**	**MCP**
BM	42.20	42.48
CJ	27.73	23.45
CW	14.57	12.63
DF	34.35	41.38
EM	12.82	13.27
EMC	16.08	24.08
JB	31.63	38.68
JC	31.7	46.52
JG	20.7	12.08
JL	17.05	9.17
KJ	5.58	23.50
KM	42.27	65.75
LM	68.93	84.67
MP	16.62	21.58
MU	8.27	6.62
AVG	**26.03**	**31.06**
SEM	**4.27**	**5.76**

Time to Exhaustion		
At or Below VT		
Subject	**CHO**	**MCP**
BM	42.20	42.48
DF	34.35	41.38
EMC	16.08	24.08
JB	31.63	38.68
JC	31.7	46.52
KM	42.27	65.75
LM	68.93	84.67
MP	16.62	21.58
AVG	**35.47**	**45.64**
SEM	**5.94**	**7.38**

Time to Exhaustion		
Above VT		
Subject	**CHO**	**MCP**
CJ	27.73	23.45
CW	14.57	12.63
EM	12.82	13.27
JG	20.7	12.08
JL	17.05	9.17
KJ	5.58	23.50
MU	8.27	6.62
AVG	**15.25**	**14.39**
SEM	**2.83**	**2.50**

Study 1	Insulin (pmol/L) - Combined Group							
	CHO				MCP			
Subject	Pre	Min 118	Min 177	End	Pre	Min 118	Min 177	End
BM	55.35	56.19	55.84	37.09	59.59	50.49	41.18	43.41
CJ	65.07	48.96	76.33	31.67	58.06	46.11	45.14	27.22
CW	101.12	82.23	85.98	76.40	111.61	101.47	97.92	75.42
DF	83.34	65.21	71.81	35.28	72.99	62.99	56.74	38.34
EM	42.02	22.64	27.15	21.53	38.68	18.33	25.35	14.86
EMC	68.82	55.84	76.67	57.78	56.12	54.87	41.88	47.43
JB	73.27	23.40	47.78	10.83	55.56	36.67	27.50	12.78
JC	90.56	100.91	80.35	42.71	141.40	116.33	75.63	58.41
JG	63.48	47.23	78.48	25.35	72.23	42.43	34.52	30.91
JL	92.92	85.63	78.96	52.37	115.22	61.25	92.09	54.59
KJ	48.48	73.62	74.59	46.25	56.46	59.24	40.98	36.48
KM	57.50	51.60	40.07	27.15	68.06	60.28	58.27	39.86
LM	58.82	61.95	87.51	42.78	74.24	47.43	50.21	39.59
MP	109.94	82.51	94.38	82.44	132.09	80.01	67.57	68.62
MU	162.93	108.62	125.57	57.37	189.46	132.79	138.90	88.55
AVG	**78.24**	**64.44**	**73.43**	**43.13**	**86.78**	**64.71**	**59.59**	**45.10**
SEM	**7.91**	**6.45**	**6.12**	**5.11**	**10.83**	**7.97**	**7.95**	**5.47**

	Insulin (pmol/L) - At or Below VT							
	CHO				MCP			
Subject	Pre	Min 118	Min 177	End	Pre	Min 118	Min 177	End
BM	55.35	56.19	55.84	37.09	59.59	50.49	41.18	43.41
DF	83.34	65.21	71.81	35.28	72.99	62.99	56.74	38.34
EMC	68.82	55.84	76.67	57.78	56.12	54.87	41.88	47.43
JB	73.27	23.40	47.78	10.83	55.56	36.67	27.50	12.78
JC	90.56	100.91	80.35	42.71	141.40	116.33	75.63	58.41
KM	57.50	51.60	40.07	27.15	68.06	60.28	58.27	39.86
LM	58.82	61.95	87.51	42.78	74.24	47.43	50.21	39.59
MP	109.94	82.51	94.38	82.44	132.09	80.01	67.57	68.62
AVG	**74.70**	**62.20**	**69.30**	**42.01**	**82.51**	**63.63**	**52.37**	**43.55**
SEM	**6.72**	**8.03**	**6.87**	**7.50**	**12.13**	**8.76**	**5.48**	**5.77**

	Insulin (pmol/L) - Above VT							
	CHO				MCP			
Subject	Pre	Min 118	Min 177	End	Pre	Min 118	Min 177	End
CJ	65.07	48.96	76.33	31.67	58.06	46.11	45.14	27.22
CW	101.12	82.23	85.98	76.40	111.61	101.47	97.92	75.42
EM	42.02	22.64	27.15	21.53	38.68	18.33	25.35	14.86
JG	63.48	47.23	78.48	25.35	72.23	42.43	34.52	30.91
JL	92.92	85.63	78.96	52.37	115.22	61.25	92.09	54.59
KJ	48.48	73.62	74.59	46.25	56.46	59.24	40.98	36.48
MU	162.93	108.62	125.57	57.37	189.46	132.79	138.90	88.55
AVG	**82.29**	**66.99**	**78.15**	**44.42**	**91.67**	**65.95**	**67.84**	**46.86**
SEM	**15.73**	**10.95**	**10.83**	**7.41**	**19.56**	**14.65**	**15.96**	**10.22**

Study 1	Glucose (mmol/L) - Combined Group							
	CHO				MCP			
Subject	Pre	Min 118	Min 177	End	Pre	Min 118	Min 177	End
BM	4.52	5.48	5.61	5.58	3.74	4.29	5.27	5.00
CJ	4.42	3.80	3.90	4.26	4.87	3.89	4.05	5.04
CW	3.94	3.48	3.85	4.49	3.90	3.21	3.38	3.81
DF	4.38	4.12	3.99	4.33	3.92	3.95	3.82	4.15
EM	3.82	3.81	3.68	4.84	4.20	3.43	3.45	4.66
EMC	4.14	4.50	4.86	5.67	4.23	3.73	3.74	5.50
JB	4.29	4.41	4.60	4.62	4.28	3.97	3.84	3.88
JC	4.19	4.03	4.31	3.87	4.64	4.01	3.91	4.29
JG	4.52	3.96	5.17	5.81	4.40	3.34	4.28	4.77
JL	4.39	4.38	4.12	5.69	4.74	4.23	4.14	4.41
KJ	4.12	4.44	4.28	4.05	3.64	3.68	3.84	3.79
KM	4.01	4.09	4.28	5.16	4.59	4.05	4.26	4.48
LM	3.97	4.72	4.97	4.80	3.91	3.91	4.30	4.26
MP	3.60	3.59	3.61	4.12	4.43	3.77	4.04	5.68
MU	4.60	4.44	5.42	4.53	4.52	4.34	5.03	4.91
AVG	**4.19**	**4.22**	**4.44**	**4.79**	**4.27**	**3.85**	**4.09**	**4.58**
SEM	**0.07**	**0.13**	**0.16**	**0.17**	**0.10**	**0.09**	**0.13**	**0.15**

	Glucose (mmol/L) - At or Below VT							
	CHO				MCP			
Subject	Pre	Min 118	Min 177	End	Pre	Min 118	Min 177	End
BM	4.52	5.48	5.61	5.58	3.74	4.29	5.27	5.00
DF	4.38	4.12	3.99	4.33	3.92	3.95	3.82	4.15
EMC	4.14	4.50	4.86	5.67	4.23	3.73	3.74	5.50
JB	4.29	4.41	4.60	4.62	4.28	3.97	3.84	3.88
JC	4.19	4.03	4.31	3.87	4.64	4.01	3.91	4.29
KM	4.01	4.09	4.28	5.16	4.59	4.05	4.26	4.48
LM	3.97	4.72	4.97	4.80	3.91	3.91	4.30	4.26
MP	3.60	3.59	3.61	4.12	4.43	3.77	4.04	5.68
AVG	**4.14**	**4.37**	**4.53**	**4.77**	**4.22**	**3.96**	**4.15**	**4.65**
SEM	**0.10**	**0.20**	**0.22**	**0.23**	**0.12**	**0.06**	**0.18**	**0.23**

	Glucose (mmol/L) - Above VT							
	CHO				MCP			
Subject	Pre	Min 118	Min 177	End	Pre	Min 118	Min 177	End
CJ	4.42	3.80	3.90	4.26	4.87	3.89	4.05	5.04
CW	3.94	3.48	3.85	4.49	3.90	3.21	3.38	3.81
EM	3.82	3.81	3.68	4.84	4.20	3.43	3.45	4.66
JG	4.52	3.96	5.17	5.81	4.40	3.34	4.28	4.77
JL	4.39	4.38	4.12	5.69	4.74	4.23	4.14	4.41
KJ	4.12	4.44	4.28	4.05	3.64	3.68	3.84	3.79
MU	4.60	4.44	5.42	4.53	4.52	4.34	5.03	4.91
AVG	**4.26**	**4.04**	**4.35**	**4.81**	**4.32**	**3.73**	**4.02**	**4.48**
SEM	**0.11**	**0.14**	**0.26**	**0.26**	**0.17**	**0.17**	**0.21**	**0.19**

Study 1	Lactate (mmol/L) - Combined Group							
	CHO				MCP			
Subject	Pre	Min 118	Min 177	End	Pre	Min 118	Min 177	End
BM	0.92	0.72	0.83	1.83	0.39	0.83	0.48	1.41
CJ	0.52	1.21	0.69	4.15	0.55	1.30	1.06	5.45
CW	0.75	1.56	1.09	2.69	0.57	1.50	1.25	4.09
DF	1.28	3.14	1.27	3.25	1.05	2.52	1.49	3.37
EM	0.66	1.47	1.12	4.57	0.74	1.59	1.37	5.15
EMC	0.88	2.27	1.82	4.36	0.85	1.96	1.58	4.35
JB	0.64	0.96	0.77	1.63	0.71	1.12	0.95	1.69
JC	1.22	1.04	1.00	2.11	1.47	1.28	0.98	2.05
JG	0.73	1.42	1.09	7.82	0.81	1.14	1.02	5.70
JL	1.27	1.38	1.07	5.50	0.84	1.34	1.02	4.22
KJ	0.68	0.99	0.90	3.36	1.03	1.53	1.06	3.56
KM	0.61	1.09	1.25	3.07	0.86	1.24	0.97	2.30
LM	0.34	1.37	1.13	1.41	0.37	1.00	0.93	1.31
MP	1.04	1.24	1.01	6.98	1.36	1.42	1.18	7.59
MU	1.17	1.17	1.00	2.25	1.47	1.23	1.25	3.76
AVG	**0.85**	**1.40**	**1.07**	**3.67**	**0.87**	**1.40**	**1.11**	**3.73**
SEM	**0.08**	**0.15**	**0.07**	**0.50**	**0.09**	**0.11**	**0.07**	**0.46**

	Lactate (mmol/L) - At or Below VT							
	CHO				MCP			
Subject	Pre	Min 118	Min 177	End	Pre	Min 118	Min 177	End
BM	0.92	0.72	0.83	1.83	0.39	0.83	0.48	1.41
DF	1.28	3.14	1.27	3.25	1.05	2.52	1.49	3.37
EMC	0.88	2.27	1.82	4.36	0.85	1.96	1.58	4.35
JB	0.64	0.96	0.77	1.63	0.71	1.12	0.95	1.69
JC	1.22	1.04	1.00	2.11	1.47	1.28	0.98	2.05
KM	0.61	1.09	1.25	3.07	0.86	1.24	0.97	2.30
LM	0.34	1.37	1.13	1.41	0.37	1.00	0.93	1.31
MP	1.04	1.24	1.01	6.98	1.36	1.42	1.18	7.59
AVG	**0.87**	**1.48**	**1.13**	**3.08**	**0.88**	**1.42**	**1.07**	**3.01**
SEM	**0.11**	**0.29**	**0.12**	**0.66**	**0.14**	**0.20**	**0.12**	**0.75**

	Lactate (mmol/L) - Above VT							
	CHO				MCP			
Subject	Pre	Min 118	Min 177	End	Pre	Min 118	Min 177	End
CJ	0.52	1.21	0.69	4.15	0.55	1.30	1.06	5.45
CW	0.75	1.56	1.09	2.69	0.57	1.50	1.25	4.09
EM	0.66	1.47	1.12	4.57	0.74	1.59	1.37	5.15
JG	0.73	1.42	1.09	7.82	0.81	1.14	1.02	5.70
JL	1.27	1.38	1.07	5.50	0.84	1.34	1.02	4.22
KJ	0.68	0.99	0.90	3.36	1.03	1.53	1.06	3.56
MU	1.17	1.17	1.00	2.25	1.47	1.23	1.25	3.76
AVG	**0.83**	**1.32**	**0.99**	**4.33**	**0.86**	**1.37**	**1.15**	**4.56**
SEM	**0.11**	**0.07**	**0.06**	**0.72**	**0.12**	**0.06**	**0.05**	**0.32**

Study 1	Myoglobin (nmol/L) - Combined Group							
	CHO				MCP			
Subject	Pre	Min 118	Min 177	End	Pre	Min 118	Min 177	End
BM	0.85	0.95	0.91	1.18	0.83	0.86	0.91	0.99
CJ	0.99	1.41	1.29	1.52	1.05	1.21	1.25	1.53
CW	0.84	0.89	0.87	0.84	0.84	0.95	0.85	0.85
DF	1.30	1.36	1.54	1.34	1.13	1.28	1.31	1.38
EM	1.29	1.32	1.40	1.75	1.27	1.27	1.29	1.36
EMC	1.04	1.08	1.04	1.15	0.85	0.88	0.86	1.02
JB	1.27	1.27	1.28	1.30	1.27	1.28	1.27	1.27
JC	0.87	0.87	0.89	0.88	0.90	0.97	0.98	0.92
JG	1.85	2.54	2.24	3.35	1.43	1.35	1.53	1.67
JL	1.31	1.31	1.35	1.49	1.27	1.44	1.43	1.37
KJ	0.68	0.67	0.70	0.73	0.82	0.67	0.61	0.65
KM	1.05	1.05	1.44	1.77	1.07	1.27	1.34	1.68
MP	0.83	1.14	1.00	1.08	0.97	0.97	1.06	1.15
MU	0.95	0.95	1.05	1.17	0.96	0.99	1.03	1.12
AVG	**1.08**	**1.20**	**1.22**	**1.40**	**1.05**	**1.10**	**1.12**	**1.21**
SEM	**0.08**	**0.11**	**0.10**	**0.17**	**0.05**	**0.06**	**0.07**	**0.08**

	Myoglobin (nmol/L) - At or Below VT							
	CHO				MCP			
Subject	Pre	Min 118	Min 177	End	Pre	Min 118	Min 177	End
BM	0.85	0.95	0.91	1.18	0.83	0.86	0.91	0.99
DF	1.30	1.36	1.54	1.34	1.13	1.28	1.31	1.38
EMC	1.04	1.08	1.04	1.15	0.85	0.88	0.86	1.02
JB	1.27	1.27	1.28	1.30	1.27	1.28	1.27	1.27
JC	0.87	0.87	0.89	0.88	0.90	0.97	0.98	0.92
KM	1.05	1.05	1.44	1.77	1.07	1.27	1.34	1.68
LM	0.96	0.98	0.98	0.93	1.02	0.98	1.02	1.02
MP	0.83	1.14	1.00	1.08	0.97	0.97	1.06	1.15
AVG	**1.02**	**1.09**	**1.13**	**1.20**	**1.00**	**1.06**	**1.09**	**1.18**
SEM	**0.06**	**0.06**	**0.09**	**0.10**	**0.05**	**0.06**	**0.07**	**0.09**

	Myoglobin (nmol/L) - Above VT							
	CHO				MCP			
Subject	Pre	Min 118	Min 177	End	Pre	Min 118	Min 177	End
CJ	0.99	1.41	1.29	1.52	1.05	1.21	1.25	1.53
CW	0.84	0.89	0.87	0.84	0.84	0.95	0.85	0.85
EM	1.29	1.32	1.40	1.75	1.27	1.27	1.29	1.36
JG	1.85	2.54	2.24	3.35	1.43	1.35	1.53	1.67
JL	1.31	1.31	1.35	1.49	1.27	1.44	1.43	1.37
KJ	0.68	0.67	0.70	0.73	0.82	0.67	0.61	0.65
MU	0.95	0.95	1.05	1.17	0.96	0.99	1.03	1.12
AVG	**1.13**	**1.30**	**1.27**	**1.55**	**1.09**	**1.13**	**1.14**	**1.22**
SEM	**0.15**	**0.23**	**0.19**	**0.33**	**0.09**	**0.10**	**0.12**	**0.14**

Study 1	RPE: Low Intensity Intervals					
	Combined Group					
	CHO			MCP		
Subject	**Min 90**	**Min 130**	**Min 161**	**Min 90**	**Min 130**	**Min 161**
BM	9	9	10	9	9	9
CJ	11	11	12	12	12	12
CW	8	11	10	9	11	11
DF	12	12	12	11	12	11
EM	11	11	11.5	11	11	11
EMC	12	11.5	11	13	12	12
JB	12	13	12	12	12	13
JC	12	14	14	12	13	13
JG	12	12	11	12	12	13
JL	9	11	9	11	11	11
KJ	11	11	11	11	11	10
KM	11.5	13	13	11	12	13
LM	11	12	13	13	14	14
MP	11.5	11.5	12	12	12	12
MU	12	12	12	12	12	13
AVG	**11**	**12**	**12**	**11**	**12**	**12**
SEM	**0.33**	**0.30**	**0.33**	**0.31**	**0.28**	**0.35**

	At or Below VT					
	CHO			MCP		
Subject	**Min 90**	**Min 130**	**Min 161**	**Min 90**	**Min 130**	**Min 161**
BM	9	9	10	9	9	9
DF	12	12	12	11	12	11
EMC	12	11.5	11	13	12	12
JB	12	13	12	12	12	13
JC	12	14	14	12	13	13
KM	11.5	13	13	11	12	13
LM	11	12	13	13	14	14
MP	11.5	11.5	12	12	12	12
AVG	**11**	**12**	**12**	**12**	**12**	**12**
SEM	**0.36**	**0.53**	**0.44**	**0.46**	**0.50**	**0.55**

	Above VT					
	CHO			MCP		
Subject #	**Min 90**	**Min 130**	**Min 161**	**Min 90**	**Min 130**	**Min 161**
CJ	11	11	12	12	12	12
CW	8	11	10	9	11	11
EM	11	11	11.5	11	11	11
JG	12	12	11	12	12	13
JL	9	11	9	11	11	11
KJ	11	11	11	11	11	10
MU	12	12	12	12	12	13
AVG	**11**	**11**	**11**	**11**	**11**	**12**
SEM	**0.57**	**0.18**	**0.41**	**0.40**	**0.20**	**0.43**

Study 1	RPE: High Intensity Intervals					
	Combined Group					
	CHO			MCP		
Subject	**Min 115**	**Min 159**	**Min 184**	**Min 115**	**Min 159**	**Min 184**
BM	13	13.5	15	13	13	15
CJ	15	15	16	14	15	18
CW	11	11	14	13	13	15
DF	15	15	16	15	14	17
EM	14	15	17	13.5	11	18
EMC	14	12	16	14	14	17
JB	16	16	17	14.5	16	15
JC	13	15	16	13	14	15.5
JG	13	12	15	12	14	15
JL	14	13	15	13	14	16
KJ	13	15	17	13	12	15
KM	13.5	13.5	14	13.5	14	15
LM	14	15	16	14	14	16
MP	13	15	18	13	15	18
MU	15	14	15	15	16	15
AVG	**14**	**14**	**16**	**14**	**14**	**16**
SEM	**0.31**	**0.37**	**0.30**	**0.22**	**0.34**	**0.32**

	RPE: High Intensity Intervals					
	At or Below VT					
	CHO			MCP		
Subject	**Min 115**	**Min 159**	**Min 184**	**Min 115**	**Min 159**	**Min 184**
BM	13	13.5	15	13	13	15
DF	15	15	16	15	14	17
EMC	14	12	16	14	14	17
JB	16	16	17	14.5	16	15
JC	13	15	16	13	14	15.5
KM	13.5	13.5	14	13.5	14	15
LM	14	15	16	14	14	16
MP	13	15	18	13	15	18
AVG	**14**	**14**	**16**	**14**	**14**	**16**
SEM	**0.38**	**0.45**	**0.42**	**0.27**	**0.31**	**0.41**

	RPE: High Intensity Intervals					
	Above VT					
	CHO			MCP		
Subject	**Min 115**	**Min 159**	**Min 184**	**Min 115**	**Min 159**	**Min 184**
CJ	15	15	16	14	15	18
CW	11	11	14	13	13	15
EM	14	15	17	13.5	11	18
JG	13	12	15	12	14	15
JL	14	13	15	13	14	16
KJ	13	15	17	13	12	15
MU	15	14	15	15	16	15
AVG	**14**	**14**	**16**	**13**	**14**	**16**
SEM	**0.53**	**0.61**	**0.43**	**0.36**	**0.65**	**0.53**

Study 1	HR (bpm): Low Intensity Intervals					
	Combined Group					
	CHO			MCP		
Subject	**Min 90**	**Min 130**	**Min 161**	**Min 90**	**Min 130**	**Min 161**
BM	106	113	117	109	114	121
CJ	125	127	138	124	130	142
CW	101	109	114	104	108	112
DF	139	151	147	143	148	145
EM	126	142	148	131	137	140
EMC	126	126	135	119	114	120
JB	135	154	151	137	140	147
JC	115	125	138	131	131	137
JG	119	121	123	113	119	134
JL	102	110	120	104	106	110
KJ	117	120	121	115	127	138
KM	122	128	134	115	129	134
LM	120	110	110	100	108	103
MP	128	138	140	121	130	128
MU	132	140	145	125	133	139
AVG	**120.9**	**127.6**	**132.1**	**119.4**	**124.9**	**130.0**
SEM	**2.9**	**3.8**	**3.5**	**3.3**	**3.3**	**3.5**

	HR (bpm): Low Intensity Intervals					
	At or Below VT					
	CHO			MCP		
Subject	**Min 90**	**Min 130**	**Min 161**	**Min 90**	**Min 130**	**Min 161**
BM	106	113	117	109	114	121
DF	139	151	147	143	148	145
EMC	126	126	135	119	114	120
JB	135	154	151	137	140	147
JC	115	125	138	131	131	137
KM	122	128	134	115	129	134
LM	120	110	110	100	108	103
MP	128	138	140	121	130	128
AVG	**123.9**	**130.6**	**134.0**	**121.9**	**126.8**	**129.4**
SEM	**3.8**	**5.7**	**5.0**	**5.1**	**4.9**	**5.2**

	HR (bpm): Low Intensity Intervals					
	Above VT					
	CHO			MCP		
Subject	**Min 90**	**Min 130**	**Min 161**	**Min 90**	**Min 130**	**Min 161**
CJ	125	127	138	124	130	142
CW	101	109	114	104	108	112
EM	126	142	148	131	137	140
JG	119	121	123	113	119	134
JL	102	110	120	104	106	110
KJ	117	120	121	115	127	138
MU	132	140	145	125	133	139
AVG	**117.4**	**124.1**	**129.9**	**116.6**	**122.9**	**130.7**
SEM	**4.5**	**5.0**	**5.1**	**4.0**	**4.6**	**5.2**

Study 1	HR (bpm): High Intensity Intervals					
	Combined Group					
	CHO			MCP		
Subject	**Min 115**	**Min 159**	**Min 184**	**Min 115**	**Min 159**	**Min 184**
BM	138	135	145	134	135	150
CJ	161	161	176	153	159	166
CW	125	130	139	125	130	152
DF	172	168	180	172	169	182
EM	163	168	185	161	158	189
EMC	138	147	156	134	135	148
JB	174	172	180	162	164	176
JC	134	140	152	143	145	156
JG	147	137	166	140	147	159
JL	132	139	157	131	131	152
KJ	151	151	168	145	151	173
KM	143	144	158	143	142	153
LM	137	137	143	107	104	139
MP	154	152	170	153	154	180
MU	161	158	168	153	153	166
AVG	**148.7**	**149.3**	**162.9**	**143.7**	**145.1**	**162.7**
SEM	**3.9**	**3.5**	**3.7**	**4.2**	**4.3**	**3.8**

	HR (bpm): High Intensity Intervals					
	At or Below VT					
	CHO			MCP		
Subject	**Min 115**	**Min 159**	**Min 184**	**Min 115**	**Min 159**	**Min 184**
BM	138	135	145	134	135	150
DF	172	168	180	172	169	182
EMC	138	147	156	134	135	148
JB	174	172	180	162	164	176
JC	134	140	152	143	145	156
KM	143	144	158	143	142	153
LM	137	137	143	107	104	139
MP	154	152	170	153	154	180
AVG	**148.8**	**149.4**	**160.5**	**143.5**	**143.5**	**160.5**
SEM	**5.7**	**4.9**	**5.2**	**7.0**	**7.2**	**5.8**

	HR (bpm): High Intensity Intervals					
	Above VT					
	CHO			MCP		
Subject	**Min 115**	**Min 159**	**Min 184**	**Min 115**	**Min 159**	**Min 184**
CJ	161	161	176	153	159	166
CW	125	130	139	125	130	152
EM	163	168	185	161	158	189
JG	147	137	166	140	147	159
JL	132	139	157	131	131	152
KJ	151	151	168	145	151	173
MU	161	158	168	153	153	166
AVG	**148.6**	**149.1**	**165.6**	**144.0**	**147.0**	**165.3**
SEM	**5.7**	**5.3**	**5.5**	**4.9**	**4.5**	**4.9**

Study 1	CHO Utilization, g/min									
	Combined Group									
	CHO					MCP				
Subject	**Min 10**	**Min 50**	**Min 130**	**Min 135**	**Min 184**	**Min 10**	**Min 50**	**Min 130**	**Min 135**	**Min 184**
BM	1.83	3.39	1.75	3.02	4.49	1.91	3.27	1.65	3.47	4.24
CJ	1.94	3.69	1.80	2.87	4.53	1.76	3.61	1.66	2.62	4.34
CW	2.12	3.64	1.87	3.36	4.83	1.55	3.07	1.23	2.31	4.53
DF	1.62	3.48	1.96	3.05	3.63	1.79	3.49	1.77	2.84	3.54
EM	1.97	3.78	1.95	3.15	4.58	2.07	3.56	1.88	3.42	4.50
EMC	1.35	2.41	1.14	2.36	2.74	1.28	2.51	1.03	1.93	2.68
JB	1.22	2.35	1.39	2.42	3.03	1.22	2.43	1.31	2.23	3.02
JC	0.92	1.66	1.26	1.39	2.15	1.09	1.82	1.22	1.60	2.14
JG	2.08	3.84	1.79	2.98	4.72	2.32	3.64	1.84	3.14	4.65
JL	1.52	3.10	1.99	3.24	4.72	1.99	3.37	1.93	3.57	4.73
KJ	0.64	1.51	0.74	1.37	2.02	0.68	1.44	0.83	1.51	2.07
KM	1.15	1.92	1.08	1.66	2.97	1.26	1.92	1.13	1.86	2.87
LM	1.37	2.57	1.35	2.09	2.51	1.22	2.38	1.36	2.07	2.47
MP	1.75	3.40	2.28	2.76	4.52	1.86	3.62	1.98	2.98	4.47
MU	1.24	2.20	1.36	2.40	3.35	1.27	2.06	1.14	2.40	3.07
AVG	**1.51**	**2.86**	**1.58**	**2.54**	**3.65**	**1.55**	**2.81**	**1.46**	**2.53**	**3.56**
SEM	**0.11**	**0.21**	**0.11**	**0.17**	**0.27**	**0.12**	**0.20**	**0.10**	**0.18**	**0.25**

Study 1	Fat Utilization, g/min									
	Combined Group									
	CHO					MCP				
Subject	**Min 10**	**Min 50**	**Min 130**	**Min 135**	**Min 184**	**Min 10**	**Min 50**	**Min 130**	**Min 135**	**Min 184**
BM	0.25	0.17	0.39	0.34	0.00	0.21	0.22	0.37	0.17	0.13
CJ	0.17	0.06	0.20	0.26	0.00	0.24	0.11	0.32	0.50	0.07
CW	0.19	0.18	0.36	0.30	0.00	0.47	0.34	0.73	0.70	0.14
DF	0.22	0.00	0.13	0.15	0.00	0.20	0.00	0.24	0.25	0.05
EM	0.22	0.00	0.26	0.22	0.00	0.19	0.11	0.31	0.17	0.00
EMC	0.12	0.00	0.22	0.04	0.00	0.14	0.00	0.23	0.17	0.00
JB	0.23	0.12	0.19	0.12	0.00	0.23	0.08	0.21	0.20	0.00
JC	0.18	0.08	0.04	0.16	0.00	0.10	0.00	0.06	0.08	0.00
JG	0.14	0.00	0.29	0.33	0.00	0.07	0.06	0.35	0.28	0.00
JL	0.40	0.28	0.22	0.22	0.00	0.18	0.17	0.26	0.11	0.00
KJ	0.12	0.02	0.10	0.09	0.00	0.11	0.07	0.07	0.05	0.00
KM	0.22	0.31	0.33	0.43	0.15	0.21	0.31	0.29	0.36	0.14
LM	0.22	0.13	0.22	0.34	0.22	0.27	0.27	0.26	0.40	0.28
MP	0.17	0.11	0.07	0.25	0.00	0.17	0.00	0.22	0.27	0.00
MU	0.28	0.25	0.19	0.16	0.00	0.24	0.28	0.30	0.21	0.15
AVG	**0.21**	**0.11**	**0.21**	**0.23**	**0.02**	**0.20**	**0.13**	**0.28**	**0.26**	**0.06**
SEM	**0.02**	**0.03**	**0.03**	**0.03**	**0.02**	**0.02**	**0.03**	**0.04**	**0.04**	**0.02**

Study 1	CHO Utilization, g/min									
	At or Below VT									
	CHO					MCP				
Subject	**Min 10**	**Min 50**	**Min 130**	**Min 135**	**Min 184**	**Min 10**	**Min 50**	**Min 130**	**Min 135**	**Min 184**
BM	1.83	3.39	1.75	3.02	4.49	1.91	3.27	1.65	3.47	4.24
DF	1.62	3.48	1.96	3.05	3.63	1.79	3.49	1.77	2.84	3.54
EMC	1.35	2.41	1.14	2.36	2.74	1.28	2.51	1.03	1.93	2.68
JB	1.22	2.35	1.39	2.42	3.03	1.22	2.43	1.31	2.23	3.02
JC	0.92	1.66	1.26	1.39	2.15	1.09	1.82	1.22	1.60	2.14
KM	1.15	1.92	1.08	1.66	2.97	1.26	1.92	1.13	1.86	2.87
LM	1.37	2.57	1.35	2.09	2.51	1.22	2.38	1.36	2.07	2.47
MP	1.75	3.40	2.28	2.76	4.52	1.86	3.62	1.98	2.98	4.47
AVG	**1.40**	**2.65**	**1.53**	**2.35**	**3.25**	**1.45**	**2.68**	**1.43**	**2.37**	**3.18**
SEM	**0.11**	**0.25**	**0.15**	**0.21**	**0.31**	**0.12**	**0.25**	**0.12**	**0.23**	**0.29**

Study 1	Fat Utilization, g/min									
	At or Below VT									
	CHO					MCP				
Subject	**Min 10**	**Min 50**	**Min 130**	**Min 135**	**Min 184**	**Min 10**	**Min 50**	**Min 130**	**Min 135**	**Min 184**
BM	0.25	0.17	0.39	0.34	0.00	0.21	0.22	0.37	0.17	0.13
DF	0.22	0.00	0.13	0.15	0.00	0.20	0.00	0.24	0.25	0.05
EMC	0.12	0.00	0.22	0.04	0.00	0.14	0.00	0.23	0.17	0.00
JB	0.23	0.12	0.19	0.12	0.00	0.23	0.08	0.21	0.20	0.00
JC	0.18	0.08	0.04	0.16	0.00	0.10	0.00	0.06	0.08	0.00
KM	0.22	0.31	0.33	0.43	0.15	0.21	0.31	0.29	0.36	0.14
LM	0.22	0.13	0.22	0.34	0.22	0.27	0.27	0.26	0.40	0.28
MP	0.17	0.11	0.07	0.25	0.00	0.17	0.00	0.22	0.27	0.00
AVG	**0.20**	**0.11**	**0.20**	**0.23**	**0.05**	**0.19**	**0.11**	**0.24**	**0.24**	**0.08**
SEM	**0.01**	**0.04**	**0.04**	**0.05**	**0.03**	**0.02**	**0.05**	**0.03**	**0.04**	**0.04**

Study 1	CHO Utilization, g/min									
	Above VT									
	CHO					MCP				
Subject	**Min 10**	**Min 50**	**Min 130**	**Min 135**	**Min 184**	**Min 10**	**Min 50**	**Min 130**	**Min 135**	**Min 184**
CJ	1.94	3.69	1.80	2.87	4.53	1.76	3.61	1.66	2.62	4.34
CW	2.12	3.64	1.87	3.36	4.83	1.55	3.07	1.23	2.31	4.53
EM	1.97	3.78	1.95	3.15	4.58	2.07	3.56	1.88	3.42	4.50
JG	2.08	3.84	1.79	2.98	4.72	2.32	3.64	1.84	3.14	4.65
JL	1.52	3.10	1.99	3.24	4.72	1.99	3.37	1.93	3.57	4.73
KJ	0.64	1.51	0.74	1.37	2.02	0.68	1.44	0.83	1.51	2.07
MU	1.24	2.20	1.36	2.40	3.35	1.27	2.06	1.14	2.40	3.07
AVG	**1.64**	**3.11**	**1.64**	**2.77**	**4.11**	**1.67**	**2.97**	**1.50**	**2.71**	**3.99**
SEM	**0.21**	**0.34**	**0.17**	**0.26**	**0.40**	**0.21**	**0.33**	**0.16**	**0.27**	**0.38**

Study 1	Fat Utilization, g/min									
	Above VT									
	CHO					MCP				
Subject	**Min 10**	**Min 50**	**Min 130**	**Min 135**	**Min 184**	**Min 10**	**Min 50**	**Min 130**	**Min 135**	**Min 184**
CJ	0.17	0.06	0.20	0.26	0.00	0.24	0.11	0.32	0.50	0.07
CW	0.19	0.18	0.36	0.30	0.00	0.47	0.34	0.73	0.70	0.14
EM	0.22	0.00	0.26	0.22	0.00	0.19	0.11	0.31	0.17	0.00
JG	0.14	0.00	0.29	0.33	0.00	0.07	0.06	0.35	0.28	0.00
JL	0.40	0.28	0.22	0.22	0.00	0.18	0.17	0.26	0.11	0.00
KJ	0.12	0.02	0.10	0.09	0.00	0.11	0.07	0.07	0.05	0.00
MU	0.28	0.25	0.19	0.16	0.00	0.24	0.28	0.30	0.21	0.15
AVG	**0.22**	**0.11**	**0.23**	**0.23**	**0.00**	**0.21**	**0.16**	**0.34**	**0.29**	**0.05**
SEM	**0.04**	**0.05**	**0.03**	**0.03**	**0.00**	**0.05**	**0.04**	**0.07**	**0.09**	**0.03**

Study 2	Subject Characteristics				
Subject	Age (yr)	Weight (kg)	Height (cm)	VO_2max (L·min)	VO_2max (ml·kg·min)
BF	37	67.6	169	3.51	51.8
BH	41	57.1	157	2.72	47.6
DF	26	64.7	171	4.01	61.9
EM	28	71.3	177	4.42	62.0
EW	29	67.1	171	3.02	44.9
JB	30	58.8	157	2.67	45.4
JCB	27	58.7	159	3.13	53.2
JP	32	82.1	182	4.08	49.7
LM	30	73.7	183	3.45	46.8
MJ	38	76.6	185	4.83	63.0
AVG	**31.8**	**67.8**	**171.1**	**3.6**	**52.6**
SEM	**1.6**	**2.6**	**3.4**	**0.2**	**2.3**

Study 2	Time Trial Data						
Time Trial Time (min)					Average Watts		
Subject	CM	CHO	PLA	Subject	CM	CHO	PLA
EM	75.7	78.72	0	EM	179	164	145
LM	74.34	79.01	78.86	LM	188	162	162
EW	78.46	86.99	82.93	EW	163	127	142
JB	91.16	104.99	97.95	JB	114	83	96
MJ	70.47	67.22	71.74	MJ	228	246	209
JCB	85.91	89.79	91.55	JCB	131	118	112
BF	83.45	97.62	100.94	BF	141	99	92
DF	78.07	80.12	89.8	DF	170	157	118
BH	84.7	92.03	91.4	BH	135	111	113
JP	72.02	80.86	77.15	JP	206	157	174
AVG	**79.43**	**85.74**	**86.92**	**AVG**	**165.50**	**142.40**	**136.30**
SEM	**2.11**	**3.44**	**3.28**	**SEM**	**11.37**	**14.62**	**11.80**
Average Heart Rate				Average RPE			
Subject	CM	CHO	PLA	Subject	CM	CHO	PLA
EM	157	147	145	EM	14.75	14.33	15
LM	141	126	133	LM	14.6	16.33	15.75
EW	204	192	201	EW	14.58	12.75	14.75
JB	152	128	148	JB	13	10.75	11.71
MJ	178	176	168	MJ	15.4	14.4	13.2
JCB	162	148	147	JCB	15.33	14.67	14.57
BF	153	128	117	BF	13.33	14.43	13.78
DF	169	159	151	DF	15.67	15.33	14.7
BH	158	125	136	BH	15.58	15	15
JP	154	130	143	JP	12.6	13.5	12
AVG	**162.8**	**145.9**	**148.9**	**AVG**	**14.48**	**14.15**	**14.05**
SEM	**5.6**	**7.4**	**7.1**	**SEM**	**0.35**	**0.49**	**0.43**

Study 2						
Insulin (pmol/L)						
CM						
Subjects	Pre	R0	R45	R120	R End	TT End
BF	10.667	7.188	31.625	14.357	12.093	3.046
BH	20.395	11.557	119.19	16.535	18.521	9.775
DF	11.139	6.759	39.337	12.556	20.481	6.937
EM	9.713	6.384	23.107	11.53	15.271	9.299
JB	8.52	8.458	33.863	21.166	24.064	6.97
JCB	13.218	5.995	48.086	18.797	12.41	5.506
JP	8.973	6.078	36.925	8.688	10.319	5.24
LM	21.878	15.623	75.459	14.846	11.756	7.18
MJ	11.05	4.16	18.07	10.421	15.246	5.669
AVE	**12.84**	**8.02**	**47.30**	**14.32**	**15.57**	**6.62**
SE	**1.64**	**1.17**	**10.54**	**1.34**	**1.53**	**0.69**
CHO						
	Pre	R0	R45	R120	R End	TT End
BF	8.482	5.96	38.172	23.463	34.502	4.063
BH	15.314	13.11	85.708	22.725	24.485	8.96
DF	13.986	11.94	84.263	14.128	54.968	7.76
EM	13.086	7.339	61.372	15.173	27.865	6.577
JB	9.989	10.171	50.245	44.769	53.049	14.192
JCB	13.444	8.129	37.902	20	13.933	4.945
JP	7.179	3.563	50.889	25.968	27.415	5.37
LM	19.716	13.97	49.415	20.502	29.209	12.331
MJ	15.774	9.459	70.384	13.523	43.71	6.415
AVE	**13.00**	**9.29**	**58.71**	**22.25**	**34.35**	**7.85**
SE	**1.31**	**1.14**	**6.01**	**3.17**	**4.55**	**1.14**
PLA						
	Pre	R0	R45	R120	R End	TT End
BF	9.29	3.161	65.787	3.929	5.548	3.99
BH	13.651	11.736	17.501	11.921	11	8.744
DF	15.529	8.353	8.77	9.063	8.931	4.262
EM	7.779	6.222	5.871	8.06	8.578	7.044
JB	15.391	21.187	13.286	13.552	13.202	6.094
JCB	14.901	9.018	8.41	9.745	10.117	8.253
JP	9.86	5.718	5.721	7.597	6.489	5
LM	11.88	9.801	9.984	8.115	8.055	8.336
MJ	13.209	7.096	6.918	8.088	8.987	6.075
AVE	**12.39**	**9.14**	**15.81**	**8.90**	**8.99**	**6.42**
SE	**0.95**	**1.72**	**6.37**	**0.91**	**0.77**	**0.60**

Study 2						
Glucose (mmol/L)						
CM						
Subjects	Pre	R0	R45	R120	R End	TT End
BF	70.65	59.15	91.28	73.39	70.66	50.22
BH	77.75	73.25	89.28	71.37	73.12	69.29
DF	81.36	80.97	76.08	78.71	73.94	61.11
EM	73.02	53.93	77.38	71.51	64.07	49.21
JB	76.25	84.05	77.17	77.67	72.28	72.18
JCB	75.20	69.77	72.39	82.69	68.70	61.27
JP	58.35	72.86	72.89	73.05	65.09	57.07
LM	86.79	90.02	60.63	61.44	57.62	62.91
MJ	77.58	63.61	72.42	82.64	83.04	46.41
AVE	**75.22**	**71.96**	**76.61**	**74.72**	**69.83**	**58.85**
SE	**2.62**	**3.94**	**3.08**	**2.22**	**2.40**	**2.98**
CHO						
	Pre	R0	R45	R120	R End	TT End
BF	74.59	56.27	116.21	90.79	67.77	40.53
BH	72.95	77.14	106.86	56.95	60.55	68.72
DF	79.50	111.00	104.59	76.29	92.71	59.02
EM	69.54	55.15	102.45	61.93	66.35	49.58
JB	77.15	84.12	110.02	92.23	96.20	68.96
JCB	73.65	61.19	78.86	101.46	45.89	58.33
JP	63.86	51.90	99.74	91.07	68.39	41.52
LM	76.04	72.54	98.23	81.87	62.19	49.36
MJ	82.28	109.35	97.88	80.53	87.66	57.82
AVE	**74.40**	**75.41**	**101.65**	**81.46**	**71.97**	**54.87**
SE	**1.81**	**7.49**	**3.47**	**4.87**	**5.57**	**3.48**
PLA						
	Pre	R0	R45	R120	R End	TT End
BF	76.17	51.76	56.66	53.69	51.11	33.27
BH	76.38	84.15	72.11	70.10	70.20	68.21
DF	80.74	76.47	66.79	72.58	72.20	57.43
EM	72.44	65.94	58.80	64.64	64.79	38.35
JB	82.62	94.72	61.41	61.29	63.35	58.15
JCB	75.46	65.90	71.53	72.76	62.10	62.03
JP	63.85	61.83	57.21	60.53	56.46	35.00
LM	68.56	78.42	68.30	66.02	65.22	65.18
MJ	88.22	83.29	74.82	82.58	83.44	73.02
AVE	**76.05**	**73.61**	**65.29**	**67.13**	**65.43**	**54.52**
SE	**2.45**	**4.44**	**2.31**	**2.83**	**3.11**	**5.02**

Study 2						
Lactate (mmol/L)						
	CM					
Subjects	Pre	R0	R45	R120	R End	TT End
BF	0.650	3.995	1.205	1.265	1.114	1.656
BH	1.582	3.121	2.040	1.273	1.016	2.845
DF	1.163	7.299	1.774	0.861	0.873	1.872
EM	0.525	3.612	0.955	0.451	0.577	1.479
JB	0.510	3.773	1.179	0.881	0.697	2.486
JCB	0.592	4.127	1.118	0.763	1.052	2.279
JP	0.502	4.272	1.428	0.874	0.911	1.859
LM	0.537	1.666	1.109	0.936	0.922	1.584
MJ	0.628	5.579	1.725	0.785	0.779	1.279
AVE	**0.74**	**4.16**	**1.39**	**0.90**	**0.88**	**1.93**
SE	**0.13**	**0.52**	**0.12**	**0.08**	**0.06**	**0.17**
	CHO					
	Pre	R0	R45	R120	R End	TT End
BF	0.574	3.423	0.743	0.586	0.778	1.549
BH	1.149	2.207	1.905	1.003	0.651	1.562
DF	1.012	7.002	1.951	1.170	1.175	2.273
EM	0.903	5.025	1.155	0.936	1.081	1.799
JB	0.565	3.105	1.179	0.836	0.885	1.008
JCB	0.462	1.732	0.720	0.643	0.510	1.285
JP	0.589	3.333	0.829	0.576	0.823	1.690
LM	0.592	2.362	1.409	1.172	0.767	1.453
MJ	0.624	6.393	1.898	1.518	1.189	2.332
AVE	**0.72**	**3.84**	**1.31**	**0.94**	**0.87**	**1.66**
SE	**0.08**	**0.63**	**0.17**	**0.11**	**0.08**	**0.14**
	PLA					
	Pre	R0	R45	R120	R End	TT End
BF	0.521	1.819	0.640	0.660	0.733	1.187
BH	0.979	2.747	0.855	0.946	0.901	1.179
DF	1.199	5.066	1.019	0.801	0.734	1.274
EM	0.531	1.115	0.718	0.492	0.774	0.787
JB	0.736	2.213	1.185	1.285	1.071	1.015
JCB	1.405	2.229	0.530	0.451	0.394	0.400
JP	0.860	3.985	1.627	1.217	0.972	1.100
LM	0.718	2.364	0.920	0.972	0.920	0.775
MJ	0.830	5.579	1.609	1.034	1.044	1.723
AVE	**0.86**	**3.01**	**1.01**	**0.87**	**0.84**	**1.05**
SE	**0.10**	**0.51**	**0.13**	**0.10**	**0.07**	**0.12**

Study 2	**Free Fatty Acids (mmol/L)**					
			CM			
Subjects	Pre	R0	R45	R120	R End	TT End
BF	0.2679	0.7811	0.0696	0.1124	0.0946	0.7896
BH	0.4323	0.9002	0.1954	0.2449	0.1708	0.8323
DF	0.2395	0.2776	0.0847	0.1644	0.0509	0.6776
EM	0.1406	0.4354	0.1343	0.3494	0.1530	0.6914
JB	0.4281	1.0939	0.4937	0.4380	0.2058	0.9206
JCB	0.4190	0.4986	0.3973	0.2657	0.2608	0.6717
JP	0.5979	0.6908	0.4664	0.4810	0.2695	0.7123
LM	0.2489	0.4131	0.1676	0.1594	0.0952	0.3041
MJ	0.1630	0.1982	0.1610	0.2842	0.1947	0.3779
AVE	**0.33**	**0.59**	**0.24**	**0.28**	**0.17**	**0.66**
SE	**0.05**	**0.10**	**0.06**	**0.04**	**0.03**	**0.07**
			CHO			
	Pre	R0	R45	R120	R End	TT End
BF	0.1406	0.4214	0.1139	0.0246	0.0811	0.5995
BH	0.3616	0.4627	0.4058	0.1009	0.0784	0.5270
DF	0.1442	0.2847	0.0545	0.1095	0.0264	0.2547
EM	0.1425	0.4230	0.1142	0.0363	0.0343	0.4729
JB	0.6390	0.7091	0.4720	0.4112	0.2571	1.0327
JCB	0.1656	0.4773	0.0837	0.1177	0.0869	0.7262
JP	0.6772	0.8640	0.2898	0.0684	0.0339	0.4704
LM	0.3662	0.4208	0.2645	0.0633	0.1152	0.3456
MJ	0.1244	0.2645	0.1384	0.1876	0.0749	0.3318
AVE	**0.31**	**0.48**	**0.22**	**0.12**	**0.09**	**0.53**
SE	**0.07**	**0.06**	**0.05**	**0.04**	**0.02**	**0.08**
			PLA			
	Pre	R0	R45	R120	R End	TT End
BF	0.4122	1.0034	0.7149	0.9445	0.5645	1.4318
BH	0.4738	0.6371	0.6580	0.6085	0.6200	0.7288
DF	0.3435	0.3150	0.3735	0.7441	0.8309	0.8398
EM	0.1767	1.2141	0.7238	0.6342	0.6563	1.2262
JB	0.5031	1.3695	0.7359	0.7935	0.8488	1.4072
JCB	0.5125	0.6782	0.6674	0.4730	0.4297	0.8867
JP	0.5126	0.7538	0.8275	0.4119	0.4871	0.8000
LM	0.1120	0.3325	0.2981	0.4012	0.5003	0.5393
MJ	0.0676	0.2007	0.8599	0.6640	0.3597	0.2868
AVE	**0.35**	**0.72**	**0.65**	**0.63**	**0.59**	**0.91**
SE	**0.06**	**0.14**	**0.06**	**0.06**	**0.06**	**0.13**

Study 2						
Glycerol (mmol/L)						
CM						
Subjects	Pre	R0	R45	R120	R End	TT End
BF	0.0591	0.2967	0.0621	0.0704	0.0527	0.3446
BH	0.1192	0.4081	0.1187	0.1211	0.0869	0.4221
DF	0.0554	0.2895	0.0592	0.0602	0.0358	0.2837
EM	0.0340	0.2238	0.0639	0.0706	0.0575	0.2876
JB	0.0814	0.4397	0.1710	0.0664	0.0414	0.3793
JCB	0.0816	0.4555	0.0702	0.0740	0.0940	0.4446
JP	0.0894	0.4482	0.1135	0.0908	0.0485	0.3613
LM	0.0801	0.3288	0.0880	0.0517	0.0400	0.2626
MJ	0.0285	0.2320	0.0732	0.0570	0.0520	0.2184
AVE	**0.0699**	**0.3469**	**0.0911**	**0.0736**	**0.0566**	**0.3338**
SE	**0.01**	**0.03**	**0.01**	**0.01**	**0.01**	**0.03**
CHO						
	Pre	R0	R45	R120	R End	TT End
BF	0.0374	0.2922	0.0522	0.0330	0.0144	0.2104
BH	0.0894	0.2856	0.1206	0.0571	0.0280	0.2688
DF	0.0320	0.2373	0.0851	0.0216	0.0191	0.1276
EM	0.0378	0.2593	0.0548	0.0320	0.0223	0.2437
JB	0.0685	0.4082	0.0996	0.0398	0.0353	0.3065
JCB	0.0317	0.2906	0.0442	0.0658	0.0378	0.2932
JP	0.1394	0.3905	0.0685	0.0466	0.0234	0.2129
LM	0.0987	0.2829	0.0969	0.0507	0.0369	0.2199
MJ	0.0288	0.2690	0.0858	0.0677	0.0421	0.2174
AVE	**0.0626**	**0.3017**	**0.0786**	**0.0460**	**0.0288**	**0.2334**
SE	**0.01**	**0.02**	**0.01**	**0.01**	**0.00**	**0.02**
PLA						
	Pre	R0	R45	R120	R End	TT End
BF	0.0921	0.4028	0.1240	0.1010	0.0986	0.3461
BH	0.0897	0.3723	0.1177	0.1379	0.1000	0.4186
DF	0.0645	0.2753	0.0906	0.1014	0.1237	0.3678
EM	0.0415	0.1724	0.1230	0.1018	0.1051	0.3014
JB	0.0753	0.4667	0.1447	0.1160	0.1046	0.4446
JCB	0.2098	0.4276	0.1229	0.0817	0.1137	0.2583
JP	0.0787	0.3541	0.1384	0.0851	0.0977	0.4000
LM	0.0335	0.2757	0.0909	0.0999	0.1319	0.3491
MJ	0.0303	0.1736	0.0709	0.0646	0.0566	0.2830
AVE	**0.0795**	**0.3245**	**0.1137**	**0.0988**	**0.1036**	**0.3521**
SE	**0.02**	**0.04**	**0.01**	**0.01**	**0.01**	**0.02**

Study 2						
Cortisol (pmol/L)						
CM						
Subjects	Pre	R0	R45	R120	R End	TT End
BF	24.95	33.49	19.20	13.86	15.34	35.81
BH	49.19	31.41	31.86	31.62	25.59	37.39
DF	9.87	20.69	14.00	7.90	7.44	30.78
EM	27.15	24.87	17.52	10.64	13.45	27.42
JB	20.42	29.89	22.99	12.62	9.55	37.61
JCB	23.29	34.49	21.95	13.11	21.89	23.28
JP	27.00	30.99	17.83	12.53	7.37	35.54
LM	23.65	23.90	16.24	14.20	12.42	21.67
MJ	32.31	43.43	32.60	25.07	11.85	44.78
AVE	**26.42**	**30.35**	**21.58**	**15.73**	**13.88**	**32.70**
SE	**3.50**	**2.25**	**2.21**	**2.53**	**2.08**	**2.50**
CHO						
	Pre	R0	R45	R120	R End	TT End
BF	23.88	23.55	17.28	25.05	17.19	47.18
BH	34.77	32.70	28.90	35.53	28.81	70.40
DF	13.31	35.62	30.09	14.12	8.89	16.91
EM	21.72	23.81	17.74	15.42	12.65	30.33
JB	17.89	23.13	25.96	17.77	16.14	36.13
JCB	23.36	13.09	15.80	8.79	10.32	31.34
JP	31.22	32.34	23.23	15.27	13.70	53.82
LM	25.52	15.85	15.88	14.05	11.72	41.68
MJ	36.33	56.78	43.15	29.89	15.88	48.92
AVE	**25.33**	**28.54**	**24.23**	**19.54**	**15.03**	**41.86**
SE	**2.54**	**4.34**	**3.00**	**2.90**	**1.95**	**5.18**
PLA						
	Pre	R0	R45	R120	R End	TT End
BF	28.90	31.54	22.33	21.36	14.97	37.84
BH	41.38	36.22	27.39	24.73	22.40	30.72
DF	13.85	28.99	19.44	11.82	5.44	16.89
EM	23.82	32.94	24.44	17.52	10.44	26.03
JB	14.12	24.45	17.63	12.25	12.29	39.56
JCB	26.44	23.28	18.11	14.82	12.79	19.57
JP	29.07	30.29	15.68	10.98	11.69	42.00
LM	21.29	16.39	13.16	11.44	12.04	22.93
MJ	36.06	67.71	39.60	35.56	18.66	49.79
AVE	**26.10**	**32.42**	**21.98**	**17.83**	**13.41**	**31.70**
SE	**3.05**	**4.83**	**2.64**	**2.73**	**1.62**	**3.74**

Study 2					
Myoglobin (nmol/L)					
CM					
Subjects	Pre	R0	R45	R End	TT End
BF	17.95	24.83	73.14	30.19	38.02
BH	13.08	17.24	15.72	12.35	19.60
DF	14.92	26.64	32.57	20.11	36.45
EM	27.32	28.08	37.53	29.48	29.37
JB	25.49	25.24	51.91	43.18	41.52
JCB	22.77	25.08	31.57	26.72	34.03
JP	28.70	28.87	39.87	29.69	41.96
LM	25.46	25.39	25.35	25.28	25.24
MJ	23.67	25.60	32.01	23.73	28.70
AVE	**22.15**	**25.22**	**37.74**	**26.75**	**32.77**
SE	**1.85**	**1.10**	**5.52**	**2.79**	**2.53**
CHO					
	Pre	R0	R45	R End	TT End
BF	10.02	14.37	15.26	13.29	23.61
BH	14.04	18.71	21.96	17.60	29.22
DF	18.84	31.76	46.79	39.46	52.02
EM	26.90	28.03	30.43	28.89	31.32
JB	25.21	25.62	25.43	25.41	27.67
JCB	23.73	25.58	26.17	28.01	26.44
JP	29.53	30.54	77.25	73.20	56.97
LM	25.64	25.81	25.66	25.61	25.23
MJ	24.47	27.51	48.13	48.85	68.68
AVE	**22.04**	**25.33**	**35.23**	**33.37**	**37.91**
SE	**2.14**	**1.84**	**6.38**	**6.11**	**5.57**
PLA					
	Pre	R0	R45	R End	TT End
BF	12.66	18.84	39.28	29.69	30.63
BH	15.72	23.61	21.16	28.00	28.45
DF	19.21	53.90	54.11	31.92	37.67
EM	31.73	35.55	68.92	40.10	38.25
JB	25.29	25.56	25.55	25.23	29.54
JCB	21.15	22.48	23.56	24.35	24.89
JP	29.48	28.65	33.86	30.74	38.00
LM	25.70	25.48	25.35	25.66	25.38
MJ	31.40	30.87	81.81	37.63	60.32
AVE	**23.59**	**29.44**	**41.51**	**30.37**	**34.79**
SE	**2.28**	**3.46**	**7.31**	**1.83**	**4.13**

Study 2					
CPK (U/L)					
CM					
Subjects	Pre	R0	R120	R End	TT End
BF	291.78	422.99	489.85	487.26	687.58
BH	45.20	59.74	40.06	39.70	52.20
DF	75.88	96.10	93.27	93.78	159.47
EM	97.31	114.17	78.91	142.61	147.40
JB	152.59	181.08	148.34	158.05	194.73
JCB	203.47	220.74	189.80	250.72	282.87
JP	150.26	182.25	159.29	148.05	183.26
LM	47.93	61.05	47.64	50.61	50.96
MJ	67.76	83.67	73.07	85.86	95.82
AVG	**125.80**	**157.97**	**146.69**	**161.85**	**206.03**
SE	**27.42**	**38.30**	**46.22**	**45.96**	**64.97**
CHO					
	Pre	R0	R120	R End	TT End
BF	50.07	52.71	50.93	48.30	75.83
BH	50.18	61.13	48.92	49.94	93.08
DF	63.09	98.66	88.99	92.31	139.32
EM	96.27	102.76	87.84	106.75	131.03
JB	119.62	141.71	139.82	131.42	175.28
JCB	96.14	126.89	124.42	106.30	117.01
JP	264.48	303.58	270.68	397.30	411.50
LM	47.94	55.69	48.80	50.87	102.42
MJ	63.25	85.93	107.13	139.85	259.28
AVG	**94.56**	**114.34**	**107.50**	**124.78**	**167.19**
SE	**22.85**	**25.81**	**23.20**	**35.97**	**35.53**
PLA					
	Pre	R0	R120	R End	TT End
BF	63.7	82.0	96.1	120.7	116.3
BH	35.8	52.3	36.3	37.3	83.3
DF	119.4	151.2	127.5	140.4	162.2
EM	776.9	880.2	652.9	703.3	655.2
JB	116.5	137.9	209.6	359.9	523.3
JCB	84.5	87.7	78.9	80.2	111.4
JP	203.7	239.1	182.4	202.4	245.0
LM	46.7	53.8	54.8	54.5	54.5
MJ	166.0	186.2	170.6	170.6	212.3
AVG	**179.25**	**207.82**	**178.79**	**207.69**	**240.39**
SE	**76.91**	**86.59**	**62.46**	**69.82**	**69.75**

Study 2				
CPK, Change from Baseline (percent)				
CM				
Subjects	R0	R120	R End	TT End
BF	0.450	0.679	0.670	1.357
BH	0.322	-0.114	-0.122	0.155
DF	0.266	0.229	0.236	1.102
EM	0.173	-0.189	0.465	0.515
JB	0.187	-0.028	0.036	0.276
JCB	0.085	-0.067	0.232	0.390
JP	0.213	0.060	-0.015	0.220
LM	0.274	-0.006	0.056	0.063
MJ	0.235	0.078	0.267	0.414
AVG	**0.24**	**0.07**	**0.20**	**0.50**
SE	**0.03**	**0.09**	**0.08**	**0.15**
CHO				
	R0	R120	R End	TT End
BF	0.053	0.017	-0.035	0.514
BH	0.218	-0.025	-0.005	0.855
DF	0.564	0.410	0.463	1.208
EM	0.067	-0.088	0.109	0.361
JB	0.185	0.169	0.099	0.465
JCB	0.320	0.294	0.106	0.217
JP	0.148	0.023	0.502	0.556
LM	0.162	0.018	0.061	1.137
MJ	0.359	0.694	1.211	3.100
AVG	**0.23**	**0.17**	**0.28**	**0.93**
SE	**0.05**	**0.08**	**0.13**	**0.29**
PLA				
	R0	R120	R End	TT End
BF	0.288	0.510	0.896	0.826
BH	0.459	0.012	0.040	1.325
DF	0.266	0.067	0.175	0.358
EM	0.133	-0.160	-0.095	-0.157
JB	0.183	0.799	2.089	3.491
JCB	0.038	-0.067	-0.051	0.318
JP	0.174	-0.105	-0.006	0.203
LM	0.152	0.173	0.167	0.167
MJ	0.122	0.028	0.028	0.279
AVG	**0.20**	**0.14**	**0.36**	**0.76**
SE	**0.04**	**0.11**	**0.24**	**0.37**

Study 2						
Plasma IL-6 (pg/mL)						
CHO						
	Pre	R0	R45	R120	R End	TT End
DF	137.39	251.52	105.01	188.99	115.47	153.87
MJ	138.57	316.80	182.83	187.64	159.00	359.90
LM	12.88	13.22	14.37	36.63	17.58	31.09
BH	6.13	8.95	39.83	69.33	125.82	4.81
BF	126.11	81.99	68.48	81.64	90.06	174.15
JP	7.70	17.44	7.50	15.10	14.84	35.18
JCB	6.34	6.22	5.04	13.45	2.60	8.56
EM	16.46	39.92	28.73	14.69	9.18	38.18
JB	43.45	100.93	74.67	111.94	68.57	68.71
AVG	**55.00**	**93.00**	**58.49**	**79.93**	**67.01**	**97.16**
SEM	**20.14**	**38.17**	**19.23**	**23.39**	**19.53**	**38.56**
CM						
	Pre	R0	R45	R120	R End	TT End
DF	31.35	148.74	50.95	74.55	60.99	107.06
MJ	158.55	179.56	155.68	174.72	136.16	301.14
LM	6.49	18.94	9.74	24.84	26.55	25.82
BH	0.00	6.17	0.79	1.66	1.66	29.64
BF	66.91	84.80	58.45	101.69	95.36	200.62
JP	5.99	11.15	15.37	26.16	23.61	15.98
JCB	3.98	14.46	4.82	3.54	0.00	11.74
EM	16.65	31.42	26.62	43.16	18.71	56.76
JB	56.43	64.41	39.77	65.59	61.47	127.78
AVG	**38.48**	**62.18**	**40.24**	**57.32**	**47.16**	**97.39**
SEM	**17.01**	**21.29**	**15.95**	**18.38**	**15.24**	**33.02**
PLA						
	Pre	R0	R45	R120	R End	TT End
DF	107.37	231.84	185.60	118.45	70.94	98.64
MJ	290.21	479.82	359.29	220.56	263.71	534.83
LM	6.73	18.99	20.36	35.45	31.08	23.31
BH	1.66	5.49	19.46	105.49	95.00	86.27
BF	53.76	69.60	58.92	65.00	53.65	124.15
JP	9.76	15.09	28.55	43.33	35.22	45.00
JCB	3.59	12.75	5.58	5.53	5.11	8.54
EM	7.08	22.33	29.85	20.21	23.89	37.16
JB	61.34	132.23	57.51	122.79	150.46	299.61
AVG	**60.17**	**109.79**	**85.01**	**81.86**	**81.00**	**139.72**
SEM	**31.19**	**52.59**	**38.69**	**22.47**	**27.10**	**57.34**

Study 2						
Plasma IL-8 (pg/mL)						
CHO						
	Pre	R0	R45	R120	R End	TT End
DF	75.82	86.90	54.07	59.23	49.02	69.90
MJ	29.17	65.78	52.93	39.19	34.23	62.04
LM	36.11	43.73	31.55	31.08	24.26	47.75
BH	29.61	32.43	19.89	24.42	31.46	28.05
BF	12.52	10.84	10.31	11.41	8.96	17.15
JP	7.20	11.13	7.25	8.69	6.78	32.24
JCB	3.73	4.17	3.24	3.75	1.77	2.88
EM	37.87	54.59	54.25	40.14	31.36	53.56
JB	30.07	70.46	51.38	47.63	36.90	53.83
AVG	**29.12**	**42.22**	**31.65**	**29.50**	**24.97**	**40.82**
SEM	**7.18**	**9.86**	**7.31**	**6.32**	**5.29**	**7.37**
CM						
	Pre	R0	R45	R120	R End	TT End
DF	36.64	83.07	46.53	45.31	46.23	26.60
MJ	23.62	30.31	36.73	34.55	28.49	51.30
LM	31.04	40.56	30.25	35.38	39.63	41.77
BH	25.17	29.68	30.03	26.34	22.04	47.12
BF	8.28	20.06	10.79	11.62	13.28	21.96
JP	12.12	15.54	11.12	15.17	10.27	9.70
JCB	3.65	6.69	4.73	3.31	2.12	3.71
EM	33.53	60.02	45.25	54.47	42.38	68.62
JB	46.60	59.46	42.80	47.04	35.84	80.39
AVG	**24.51**	**38.38**	**28.69**	**30.35**	**26.69**	**39.02**
SEM	**4.73**	**8.26**	**5.35**	**5.84**	**5.21**	**8.63**
PLA						
	Pre	R0	R45	R120	R End	TT End
DF	55.67	85.34	71.00	57.56	47.51	42.93
MJ	37.59	60.28	54.68	38.30	36.86	54.89
LM	29.92	39.67	28.85	33.44	33.02	33.55
BH	28.83	28.77	29.89	29.30	29.00	27.15
BF	6.27	14.40	9.12	7.64	7.50	14.64
JP	10.89	9.56	13.83	10.20	9.47	10.00
JCB	3.50	4.95	3.51	2.59	3.12	3.70
EM	28.59	53.42	78.25	67.27	65.00	92.40
JB	61.20	78.74	49.67	66.56	76.45	166.19
AVG	**29.16**	**41.68**	**37.64**	**34.76**	**34.21**	**30.52**
SEM	**6.79**	**9.90**	**9.04**	**8.34**	**8.53**	**17.14**

Study 2						
Plasma IL-10 (pg/mL)						
CHO						
	Pre	R0	R45	R120	R End	TT End
DF	5.06	27.86	39.09	8.19	6.94	26.34
MJ	138.57	316.80	182.83	187.64	159.00	359.90
LM	12.88	13.22	14.37	36.63	17.58	31.09
BH	6.13	8.95	39.83	69.33	125.82	4.81
BF	126.11	81.99	68.48	81.64	90.06	174.15
JP	7.70	17.44	7.50	15.10	14.84	35.18
JCB	12.40	9.72	9.79	9.45	4.82	39.84
EM	273.72	847.87	879.08	879.13	586.27	534.78
JB	535.08	1003.19	785.08	849.65	683.61	773.24
AVG	**124.18**	**258.56**	**225.11**	**237.41**	**187.66**	**219.92**
SEM	**59.86**	**130.82**	**116.35**	**119.95**	**86.92**	**92.27**
CM						
	Pre	R0	R45	R120	R End	TT End
DF	4.75	17.46	18.69	8.18	6.31	77.83
MJ	158.55	179.56	155.68	174.72	136.16	301.14
LM	6.49	18.94	9.74	24.84	26.55	25.82
BH	0.00	6.17	0.79	1.66	1.66	29.64
BF	66.91	84.80	58.45	101.69	95.36	200.62
JP	5.99	11.15	15.37	26.16	23.61	15.98
JCB	9.44	23.14	20.42	10.84	2.70	14.98
EM	139.35	601.81	390.86	361.51	323.96	733.65
JB	812.58	979.61	900.32	705.19	644.11	1180.03
AVG	**133.78**	**213.63**	**174.48**	**157.20**	**140.04**	**286.63**
SEM	**87.28**	**114.98**	**99.87**	**78.88**	**71.90**	**135.93**
PLA						
	Pre	R0	R45	R120	R End	TT End
DF	5.06	40.61	33.33	10.05	6.93	6.94
MJ	290.21	479.82	359.29	220.56	263.71	534.83
LM	6.73	18.99	20.36	35.45	31.08	23.31
BH	1.66	5.49	19.46	105.49	90.00	86.27
BF	53.76	69.60	58.92	65.00	53.65	124.15
JP	9.76	15.09	28.55	43.33	35.22	40.00
JCB	10.32	23.46	18.05	10.49	9.27	11.53
EM	147.62	260.19	384.71	529.20	319.94	534.20
JB	935.57	1464.41	775.19	1045.86	1049.48	1949.17
AVG	**162.30**	**264.18**	**188.65**	**229.49**	**206.59**	**367.82**
SEM	**101.89**	**159.08**	**88.52**	**115.90**	**111.96**	**210.10**

Study 2						
Plasma IL-1Ra (pg/mL)						
CHO						
	Pre	R0	R45	R120	R End	TT End
DF	59.35	55.25	42.66	8.20	51.78	68.93
MJ	36.36	92.88	39.70	30.84	17.35	34.38
LM	136.22	126.68	96.23	65.44	76.16	67.12
BH	128.44	107.28	78.07	31.47	90.44	62.92
BF	57.24	98.83	100.14	54.12	27.20	78.11
JP	71.36	80.37	89.97	55.69	73.04	73.14
JCB	56.44	45.81	40.69	39.97	24.63	24.62
EM	62.29	71.22	37.55	14.90	33.20	27.34
JB	20.79	28.59	72.46	26.31	37.99	70.43
AVG	**69.83**	**78.54**	**66.38**	**36.32**	**47.97**	**56.33**
SEM	**12.84**	**10.49**	**8.76**	**6.41**	**8.72**	**11.18**
CM						
	Pre	R0	R45	R120	R End	TT End
DF	51.03	79.33	44.81	15.52	53.73	18.35
MJ	55.02	71.40	74.55	49.76	48.23	75.31
LM	99.17	113.63	60.15	97.93	83.31	73.37
BH	117.91	134.52	100.51	26.06	100.51	95.46
BF	76.58	106.39	65.91	53.48	55.93	28.54
JP	66.76	48.09	56.33	17.72	54.62	23.66
JCB	52.01	72.17	34.78	21.52	11.30	34.07
EM	44.54	46.49	94.89	101.35	70.73	53.61
JB	44.07	103.75	72.97	25.11	19.07	86.40
AVG	**67.45**	**86.19**	**67.21**	**45.38**	**55.27**	**54.31**
SEM	**8.64**	**10.07**	**7.16**	**11.17**	**9.40**	**9.74**
PLA						
	Pre	R0	R45	R120	R End	TT End
DF	48.27	62.41	41.50	28.90	44.61	37.41
MJ	43.45	77.63	24.40	44.39	62.49	42.05
LM	91.12	109.59	80.74	80.41	107.60	80.32
BH	96.51	106.88	91.40	64.02	74.00	51.65
BF	36.94	74.69	84.21	55.32	78.30	114.50
JP	86.89	83.18	64.48	29.62	62.63	75.20
JCB	51.53	71.14	41.35	26.75	52.05	44.42
EM	54.65	47.30	23.06	54.12	27.05	32.32
JB	78.42	79.16	84.53	65.87	64.27	45.49
AVG	**65.31**	**79.11**	**59.52**	**49.93**	**63.66**	**58.15**
SEM	**7.60**	**6.55**	**9.09**	**6.29**	**7.54**	**8.90**

Study 2						
Plasma TNFα (pg/mL)						
CHO						
	Pre	R0	R45	R120	R End	TT End
DF	4.45	5.35	3.70	3.98	4.32	4.62
MJ	3.61	7.39	5.75	3.70	2.92	3.98
LM	23.71	18.73	11.10	10.73	3.48	16.27
BH	24.32	17.68	5.11	7.57	14.08	7.56
BF	4.36	7.08	4.22	2.97	3.37	3.98
JP	8.40	10.07	7.23	6.48	6.53	9.03
JCB	6.71	5.81	5.12	4.70	3.86	4.03
EM	7.52	10.26	6.54	4.41	5.02	4.45
JB	2.52	2.71	3.68	2.26	2.52	4.43
AVG	**9.51**	**9.45**	**5.82**	**5.20**	**5.12**	**6.48**
SEM	**2.81**	**1.83**	**0.77**	**0.88**	**1.19**	**1.36**
CM						
	Pre	R0	R45	R120	R End	TT End
DF	3.42	6.03	2.87	1.96	3.62	2.34
MJ	4.41	4.71	4.58	3.02	3.48	5.29
LM	18.82	15.91	8.90	12.68	14.59	15.69
BH	16.25	19.20	14.38	6.55	12.51	17.14
BF	6.17	8.15	4.69	5.15	5.63	3.58
JP	8.78	6.36	3.86	4.14	5.62	3.01
JCB	6.61	8.71	5.33	4.77	4.21	5.16
EM	6.03	6.77	5.93	5.27	6.29	5.42
JB	3.46	7.27	5.45	2.41	1.96	4.97
AVG	**8.21**	**9.23**	**6.22**	**5.10**	**6.43**	**6.95**
SEM	**1.86**	**1.64**	**1.16**	**1.07**	**1.43**	**1.83**
PLA						
	Pre	R0	R45	R120	R End	TT End
DF	4.41	6.03	4.43	2.39	2.99	3.19
MJ	3.50	5.92	4.35	3.42	3.70	3.76
LM	16.40	18.77	16.79	13.23	17.58	16.78
BH	16.10	18.40	12.29	10.05	10.70	8.70
BF	4.34	11.30	4.52	3.62	5.41	6.67
JP	9.73	3.55	6.19	4.74	6.23	6.50
JCB	7.05	8.15	5.46	4.95	5.55	5.67
EM	6.90	6.05	5.48	3.95	3.94	5.29
JB	4.92	3.89	4.66	3.25	3.49	4.26
AVG	**8.15**	**9.12**	**7.13**	**5.51**	**6.62**	**6.76**
SEM	**1.65**	**1.95**	**1.46**	**1.22**	**1.57**	**1.37**

Average CHO g/min for Recovery Period (based on the averages of 4 8-min collection periods)										
PLA										
	CHO, g/min					**Fat, g/min**				
Subjects	**R30**	**R50**	**R90**	**R210**	**AVG**	**R30**	**R50**	**R90**	**R210**	**AVG**
BF	0.057	0.105	0.096	0.090	**0.087**	0.122	0.091	0.090	0.106	**0.102**
BH	0.106	0.029	0.153	0.059	**0.087**	0.093	0.109	0.066	0.102	**0.093**
DF	-0.058	0.066	0.032	0.252	**0.073**	0.172	0.100	0.102	0.010	**0.096**
EM	0.290	0.132	0.220	0.209	**0.213**	0.095	0.088	0.071	0.090	**0.086**
EW	0.018	0.008	0.009	0.044	**0.020**	0.112	0.092	0.131	0.077	**0.103**
JB	-0.012	0.037	0.077	0.092	**0.048**	0.110	0.098	0.072	0.072	**0.088**
JCB	0.060	0.069	0.092	0.079	**0.075**	0.104	0.095	0.072	0.062	**0.083**
JP	-0.080	-0.047	0.032	0.080	**-0.004**	0.190	0.224	0.101	0.108	**0.156**
LM	0.079	0.027	0.075	0.038	**0.055**	0.118	0.127	0.102	0.115	**0.116**
MJ	0.090	0.138	0.163	0.153	**0.136**	0.098	0.101	0.096	0.086	**0.095**
CHO										
	CHO, g/min					**Fat, g/min**				
	R30	**R50**	**R90**	**R210**	**AVG**	**R30**	**R50**	**R90**	**R210**	**AVG**
BF	0.064	0.189	0.169	0.243	**0.166**	0.060	0.129	0.061	0.039	**0.072**
BH	0.164	0.209	0.267	0.311	**0.238**	0.045	0.069	0.041	0.009	**0.041**
DF	0.013	0.237	0.144	0.145	**0.135**	0.061	0.137	0.088	0.049	**0.084**
EM	0.257	0.227	0.161	0.320	**0.241**	0.074	0.072	0.093	0.049	**0.072**
EW	0.190	0.161	0.251	0.225	**0.206**	0.023	0.053	0.025	0.018	**0.030**
JB	-0.035	0.049	0.057	0.151	**0.055**	0.085	0.129	0.079	0.048	**0.085**
JCB	0.170	0.153	0.211	0.194	**0.182**	0.045	0.073	0.046	0.035	**0.050**
JP	-0.047	0.080	0.221	0.145	**0.100**	0.114	0.214	0.078	0.108	**0.129**
LM	0.153	0.193	0.148	0.237	**0.183**	0.047	0.076	0.063	0.036	**0.055**
MJ	0.239	0.382	0.381	0.307	**0.327**	0.026	0.078	0.019	0.039	**0.040**

CM										
	CHO, g/min					Fat, g/min				
	R30	**R50**	**R90**	**R210**	**AVG**	**R30**	**R50**	**R90**	**R210**	**AVG**
BF	-0.016	0.132	0.200	0.167	**0.121**	0.179	0.112	0.099	0.097	**0.122**
BH	0.250	0.182	0.232	0.154	**0.205**	0.066	0.063	0.082	0.061	**0.068**
DF	0.114	0.150	0.212	0.256	**0.183**	0.124	0.078	0.072	0.057	**0.083**
EM	0.194	0.205	0.210	0.244	**0.213**	0.130	0.067	0.104	0.092	**0.098**
EW	0.049	0.159	0.088	0.229	**0.131**	0.148	0.078	0.119	0.048	**0.098**
JB	0.155	0.139	-0.001	0.087	**0.095**	0.077	0.079	0.130	0.081	**0.092**
JCB	0.077	0.089	0.126	0.132	**0.106**	0.117	0.099	0.094	0.070	**0.095**
JP	-0.016	0.079	0.143	0.223	**0.107**	0.189	0.134	0.115	0.051	**0.123**
LM	0.213	0.179	0.185	0.219	**0.199**	0.069	0.067	0.069	0.062	**0.067**
MJ	0.222	0.210	0.340	0.159	**0.233**	0.083	0.104	0.062	0.079	**0.082**

Average CHO kcals/min for Recovery Period (based on the averages of 4 8-min collection periods)										
PLA										
	CHO, kcals/min					**Fat, kcals/min**				
Subjects	**R30**	**R50**	**R90**	**R210**	**AVG**	**R30**	**R50**	**R90**	**R210**	**AVG**
BF	0.443	0.239	0.402	0.378	**0.365**	0.829	1.112	0.961	0.821	**0.931**
BH	0.121	0.445	0.642	0.249	**0.364**	0.990	0.849	0.929	0.602	**0.842**
DF	0.276	-0.242	0.135	1.057	**0.307**	0.910	1.568	0.087	0.924	**0.872**
EM	0.556	1.217	0.922	0.877	**0.893**	0.804	0.861	0.818	0.648	**0.783**
EW	0.033	0.075	0.039	0.185	**0.083**	0.837	1.018	0.698	1.195	**0.937**
JB	0.154	-0.052	0.324	0.388	**0.203**	0.890	1.004	0.659	0.656	**0.802**
JCB	0.292	0.254	0.388	0.333	**0.317**	0.865	0.945	0.568	0.659	**0.759**
JP	-0.196	-0.338	0.133	0.338	**-0.016**	2.036	1.728	0.985	0.919	**1.417**
LM	0.114	0.330	0.317	0.158	**0.230**	1.158	1.075	1.047	0.929	**1.052**
MJ	0.581	0.378	0.684	0.641	**0.571**	0.922	0.891	0.785	0.876	**0.869**
CHO										
	CHO, kcals/min					**Fat, kcals/min**				
	R30	**R50**	**R90**	**R210**	**AVG**	**R30**	**R50**	**R90**	**R210**	**AVG**
BF	0.793	0.269	0.709	1.022	**0.698**	0.546	1.178	0.554	0.355	**0.658**
BH	0.877	0.689	1.121	1.306	**0.998**	0.407	0.628	0.374	0.079	**0.372**
DF	0.995	0.053	0.606	0.608	**0.565**	0.553	1.249	0.800	0.445	**0.762**
EM	0.952	1.077	0.677	1.345	**1.013**	0.669	0.658	0.842	0.446	**0.654**
EW	0.675	0.796	1.053	0.944	**0.867**	0.210	0.480	0.232	0.162	**0.271**
JB	0.206	-0.149	0.241	0.634	**0.233**	0.777	1.171	0.722	0.436	**0.776**
JCB	0.643	0.713	0.886	0.814	**0.764**	0.411	0.660	0.419	0.320	**0.453**
JP	0.334	-0.196	0.928	0.610	**0.419**	1.037	1.946	0.712	0.983	**1.170**
LM	0.812	0.644	0.621	0.994	**0.768**	0.424	0.691	0.576	0.324	**0.504**
MJ	1.605	1.003	1.598	1.291	**1.374**	0.234	0.707	0.172	0.358	**0.368**
CM										
	CHO, kcals/min					**Fat, kcals/min**				
	R30	**R50**	**R90**	**R210**	**AVG**	**R30**	**R50**	**R90**	**R210**	**AVG**
BF	-0.069	0.556	0.840	0.700	**0.507**	1.630	1.021	0.903	0.884	**1.110**

BH	1.050	0.766	0.973	0.649	**0.859**	0.600	0.571	0.742	0.557	**0.618**
DF	0.478	0.632	0.889	1.075	**0.769**	1.125	0.708	0.659	0.516	**0.752**
EM	0.816	0.863	0.881	1.027	**0.897**	1.180	0.605	0.949	0.833	**0.892**
EW	0.207	0.666	0.370	0.963	**0.551**	1.348	0.714	1.083	0.433	**0.895**
JB	0.650	0.583	-0.004	0.366	**0.399**	0.696	0.723	1.185	0.739	**0.836**
JCB	0.325	0.372	0.529	0.552	**0.445**	1.067	0.899	0.855	0.635	**0.864**
JP	-0.067	0.330	0.601	0.935	**0.450**	1.723	1.224	1.049	0.465	**1.115**
LM	0.893	0.751	0.779	0.921	**0.836**	0.628	0.606	0.629	0.561	**0.606**
MJ	0.934	0.881	1.428	0.669	**0.978**	0.758	0.948	0.562	0.718	**0.747**

Study 2	p-mTOR, % of standard								
	PLA			CM			CHO		
Subject	R0	R45	R End	RO	R45	R End	RO	R45	R End
BF	87.6	74.8	87.6	66.8	58.7	41.1	62.8	56.9	72.1
BH	79.3	132.1	52.0	78.2	215.3	111.8	107.8	101.9	76.6
DF	57.4	79.9	88.8	51.1	150.2	126.1	95.8	122.9	96.3
EM	62.3	34.8	59.1	41.9	94.6	62.0	51.4	86.4	67.4
EW	116.2	68.5	69.1	105.1	389.6	112.0	17.7	202.6	111.5
JB	46.1	59.9	58.9	54.7	101.0	74.2	52.6	81.8	56.3
JCB	74.6	70.3	71.6	134.5	374.6	142.4	126.0	357.2	171.0
JP	73.7	84.4	74.6	67.9	149.5	109.9	53.3	88.8	94.3
LM	72.5	67.3	67.9	72.0	112.0	84.5	54.3	130.1	73.7
MJ	62.1	64.6	61.0	48.3	148.4	81.0	74.9	84.3	80.1
AVG	**73.2**	**73.7**	**69.1**	**72.0**	**179.4**	**94.5**	**69.6**	**131.3**	**89.9**
SEM	**6.1**	**7.8**	**3.8**	**9.0**	**36.3**	**9.9**	**10.1**	**28.1**	**10.3**

Study 2	p-rpS6, % of standard								
	PLA			CM			CHO		
Subject	R0	R45	R End	RO	R45	R End	RO	R45	R End
BF	12.8	7.3	7.6	6.4	26.6	7.5	5.8	9.7	9.0
BH	6.1	12.2	10.2	6.9	42.7	5.7	6.4	35.5	4.5
DF	7.0	12.5	5.4	7.7	49.7	17.8	23.8	38.0	11.4
EM	7.4	17.2	6.4	4.3	48.2	24.2	6.0	43.3	5.0
EW	1.5	13.0	2.2	8.5	48.4	34.5	5.0	18.6	24.3
JB	4.3	31.1	22.3	4.3	16.6	22.3	4.0	4.8	2.5
JCB	6.3	9.2	2.4	7.1	17.7	8.0	5.9	34.0	6.7
JP	6.2	4.3	13.7	4.9	39.5	12.8	5.6	24.5	3.8
LM	4.4	14.6	7.0	4.5	16.3	14.3	5.9	14.3	9.9
MJ	9.5	31.7	6.7	20.1	104.5	21.3	7.7	107.4	37.1
AVG	**6.5**	**15.3**	**8.4**	**7.5**	**41.0**	**16.8**	**7.6**	**33.0**	**11.4**
SEM	**1.0**	**2.9**	**1.9**	**1.5**	**8.3**	**2.8**	**1.8**	**9.2**	**3.5**

Study 2	p-eIF2Bε, % of standard								
	PLA			CM			CHO		
Subject	R0	R45	R End	RO	R45	R End	RO	R45	R End
BF	76.5	80.3	82.4	74.8	65.7	64.7	62.0	68.1	72.0
BH	61.5	49.6	43.9	55.7	47.8	52.9	56.6	47.4	54.9
DF	76.2	91.9	86.9	75.2	105.2	86.8	82.1	79.5	84.1
EM	79.2	84.0	78.3	78.3	70.6	96.2	77.7	94.2	86.2
EW	61.5	86.9	68.9	82.2	90.4	92.1	73.3	81.0	79.9
JB	70.1	93.9	89.9	65.3	70.7	76.3	83.1	91.4	84.2
JCB	86.4	67.3	67.1	61.9	70.3	72.2	72.9	80.1	81.9
JP	69.0	70.0	67.0	64.2	70.6	80.3	57.0	64.4	64.1
LM	84.7	76.0	70.4	81.9	70.7	94.2	59.9	52.9	44.2
MJ	48.4	43.1	38.6	49.8	37.5	41.2	51.0	45.7	40.3
AVG	**71.4**	**74.3**	**69.3**	**68.9**	**69.9**	**75.7**	**67.6**	**70.5**	**69.2**
SEM	**3.7**	**5.4**	**5.4**	**3.5**	**6.0**	**5.8**	**3.7**	**5.6**	**5.5**

Study 2	p-FOXO3A, % of standard								
	PLA			CM			CHO		
Subject	R0	R45	R End	RO	R45	R End	RO	R45	R End
BF	96.3	84.6	92.9	73.8	84.3	77.8	87.2	90.9	95.3
BH	62.9	79.8	63.3	74.3	85.1	69.5	64.7	90.3	72.6
DF	41.4	58.0	50.4	55.4	68.2	54.8	66.1	74.2	50.0
EM	79.6	81.3	83.6	70.1	96.7	69.2	69.9	107.0	78.5
EW	61.8	42.9	58.3	55.1	75.4	58.1	62.9	88.7	83.0
JB	81.7	83.6	72.9	123.0	132.4	125.0	81.5	102.4	89.4
JCB	48.0	47.7	46.9	62.4	71.1	66.0	64.0	82.5	70.7
JP	42.4	57.1	55.0	41.1	57.1	52.6	42.9	55.6	58.0
LM	102.2	93.2	98.1	85.8	100.9	98.2	83.5	88.4	98.3
MJ	63.2	64.0	62.2	54.4	75.6	50.0	63.7	74.4	74.5
AVG	**67.9**	**69.2**	**68.4**	**69.5**	**84.7**	**72.1**	**68.6**	**85.4**	**77.0**
SEM	**6.8**	**5.5**	**5.6**	**7.2**	**6.7**	**7.4**	**4.1**	**4.7**	**4.9**

Study 2	Total ubiquitination, % of standard								
	PLA			CM			CHO		
Subject	R0	R45	R End	RO	R45	R End	RO	R45	R End
BF	62.8	146.0	65.6	57.2	173.1	50.8	55.1	57.6	55.9
BH	66.8	64.1	63.3	82.1	69.2	90.6	150.7	77.7	65.9
DF	92.8	81.5	104.7	84.0	82.8	87.8	84.4	116.1	163.8
EM	108.1	119.5	101.2	107.1	163.1	188.2	109.0	175.3	119.8
EW	90.3	90.8	83.2	100.5	142.5	146.9	129.7	144.5	224.8
JB	63.9	168.9	79.4	164.7	71.1	80.6	68.7	71.8	137.2
JCB	77.3	119.1	82.8	109.1	106.0	90.8	83.4	266.9	202.5
JP	92.1	112.3	92.9	127.6	106.4	133.7	93.3	89.0	96.5
LM	235.4	87.6	96.5	216.2	89.2	133.9	105.4	154.6	85.4
MJ	109.7	98.6	202.3	117.9	98.1	125.1	85.2	108.1	100.4
AVG	**99.9**	**108.8**	**97.2**	**116.6**	**110.1**	**112.8**	**96.5**	**126.2**	**125.2**
SEM	**16.0**	**9.9**	**12.5**	**14.3**	**11.7**	**12.6**	**9.0**	**19.8**	**17.9**

% change in total ubiquitination									
Study 2	PLA			CM			CHO		
Subject	R0 to R45	R45 to R End	RO to R End	R0 to R45	R45 to R End	RO to R End	R0 to R45	R45 to R End	RO to R End
BF	132.5	-55.0	4.6	202.7	-70.6	-11.1	4.6	-2.9	1.5
BH	-3.9	-1.3	-5.1	-15.8	31.0	10.3	-48.4	-15.2	-56.3
DF	-12.2	28.4	12.8	-1.4	6.1	4.5	37.7	41.0	94.1
EM	10.6	-15.3	-6.4	52.3	15.4	75.7	60.8	-31.7	9.9
EW	0.6	-8.4	-7.9	41.8	3.1	46.2	11.4	55.6	73.3
JB	164.2	-53.0	24.1	-56.8	13.4	-51.1	4.6	91.1	99.9
JCB	54.1	-30.4	7.2	-2.8	-14.3	-16.7	220.0	-24.1	142.8
JP	22.0	-17.3	0.9	-16.6	25.7	4.8	-4.7	8.5	3.4
LM	-62.8	10.2	-59.0	-58.8	50.1	-38.1	46.7	-44.8	-19.0
MJ	-10.1	105.2	84.4	-16.8	27.6	6.1	26.9	-7.2	17.8
AVG	**29.5**	**-3.7**	**5.5**	**12.8**	**8.7**	**3.1**	**36.0**	**7.0**	**36.8**
SEM	**22.0**	**14.6**	**11.2**	**23.9**	**10.4**	**11.7**	**22.6**	**13.6**	**19.7**

Study 3

Subject	Treatment	Train Group	Age (yr)	Height (cm)	Baseline Weight (kg)	Base VO_2max (L/min)	Base VO_2max (ml/kg/min)	Gender
ALL SUBJECTS								
AVG			**22.0**	**168.6**	**71.7**	**27.5**	**11.0**	M=16
SEM			**0.5**	**1.5**	**2.4**	**3.0**	**2.4**	F=16
CM								
CG	1	4	19	176.0	75.96	41.5	3.17	M
DIT	1	3	19.3	166.1	55.75	2.15	38.00	F
EV	1	4	20.8	159.3	62.4	37.8	2.42	F
JF	1	2	22.8	172.0	67	38.90	2.63	M
MF	1	3	26.1	157.5	51.1	1.86	35.35	F
MP	1	3	24.5	162.6	63	1.63	25.40	F
NG	1	4	20.7	168.1	81.3	42.0	3.48	M
PB	1	2	22.2	179.6	64.2	35.35	2.32	M
PD	1	1	24.4	181.1	113.9	41.70	4.78	M
VE	1	3	22.9	168.4	74.4	2.54	33.96	F
YL	1	1	20	169.7	70.8	35.45	2.55	M
AVG			**22.1**	**169.1**	**70.9**	**25.5**	**14.0**	
SEM			**0.7**	**2.3**	**5.1**	**5.7**	**4.7**	
CHO								
CF	2	1	19.4	178.6	81.34	47.70	3.90	M
GM	2	2	20.2	157.5	67.5	28.60	1.97	F
JQ	2	4	22.8	149.9	51.9	34.5	1.81	F
KH	2	2	20.6	171.5	67.1	27.05	1.84	M
LM	2	4	29.2	163.3	65.4	36.2	2.41	F
MM	2	2	22.8	170.2	84	39.80	3.40	M
NF	2	4	18.8	175.3	60.53	46.4	2.85	M
RC	2	1	18.9	178.6	83.6	38.05	3.21	M
RM	2	4	22.8	168.1	80.4	37.9	3.04	M
SB	2	3	20.6	163.8	69.7	2.04	28.70	F
TR	2	4	18.3	171.7	71.76	27.9	2.04	F
AVG			**21.3**	**168.0**	**71.2**	**33.3**	**5.0**	
SEM			**0.9**	**2.7**	**3.1**	**3.7**	**2.4**	
PLA								
AR	3	3	21.4	150.1	58.2	1.70	28.90	F
BM	3	3	19.4	169.7	70.31	2.50	34.65	F
DT	3	2	22.1	180.3	99.1	33.30	3.30	M
JH	3	4	20	175.8	73.8	52.3	3.85	M
JL	3	3	28.5	165.1	61.3	1.91	30.65	F
JM	3	2	26.3	180.3	97.9	36.20	3.57	M
JO	3	1	24.9	164.6	75.6	30.77	2.35	F
JW	3	4	22.8	177.3	66	38.0	2.59	M
MN	3	1	18.8	158.2	64.4	35.85	2.34	F
VR	3	3	21.3	166.4	65.4	2.11	31.70	F
AVG			**22.6**	**168.8**	**73.2**	**23.5**	**14.4**	
SEM			**1.0**	**3.1**	**4.5**	**6.1**	**4.7**	
Treatments: 1=CM; 2=CHO; 3=PLA								

Study 3	Performance and Cardiovascular Fitness Measures					
CM						
	Absolute VO_2max (L/min)			**Relative VO_2max (mL/kg/min)**		
Subject	**Base**	**Mid**	**End**	**Base**	**Mid**	**End**
CG	3.17	3.48	3.20	41.5	46.6	43.1
DIT	2.15	2.31	2.60	38.00	40.50	45.80
EV	2.42	2.50	2.71	37.8	39.0	41.3
JF	2.63	2.98	3.25	38.90	44.05	48.30
MF	1.86	2.10	2.23	35.35	39.55	43.50
MP	1.63	1.63	2.01	25.40	25.20	31.65
NG	3.48	3.60	3.81	42.0	43.5	45.5
PB	2.32	2.50	2.71	35.35	38.45	41.40
PD	4.78	4.81	5.00	41.70	42.50	44.00
VE	2.54	2.65	2.75	33.96	34.50	37
YL	2.55	2.84	2.95	35.45	40.05	41.90
AVE	**2.68**	**2.85**	**3.02**	**36.85**	**39.44**	**42.13**
SE	**0.26**	**0.26**	**0.25**	**1.43**	**1.73**	**1.37**
CHO						
	Absolute VO_2max (L/min)			**Relative VO_2max (mL/kg/min)**		
Subject	**Base**	**Mid**	**End**	**Base**	**Mid**	**End**
CF	3.90	3.86	3.96	47.70	47.07	48.75
GM	1.97	2.04	1.99	28.60	29.65	29.05
JQ	1.81	1.84	1.75	34.5	35.3	33.2
KH	1.84	2.39	2.26	27.05	33.85	31.70
LM	2.41	2.67	2.62	36.2	41.2	40.5
MM	3.40	3.51	3.55	39.80	42.20	42.80
NF	2.85	2.82	2.87	46.4	45.8	46.5
RC	3.21	3.45	3.62	38.05	41.40	42.95
RM	3.04	3.17	3.36	37.9	39.5	42.4
TR	2.04	2.14	2.20	27.9	28.7	29.5
AVE	**2.64**	**2.79**	**2.82**	**36.41**	**38.45**	**38.73**
SE	**0.23**	**0.22**	**0.24**	**2.28**	**2.01**	**2.29**
PLA						
	Absolute VO_2max (L/min)			**Relative VO_2max (mL/kg/min)**		
Subject	**Base**	**Mid**	**End**	**Base**	**Mid**	**End**
AR	1.70	1.81	1.79	28.90	30.15	29.90
BM	2.50	2.69	2.72	34.65	38.05	39.50
DT	3.30	3.37	3.55	33.30	33.60	35.20
JH	3.85	3.91	4.10	52.3	53.5	55.9
JL	1.91	2.13	2.29	30.65	34.35	36.65
JM	3.57	3.62	3.77	36.20	36.50	38.70
JO	2.35	2.37	2.37	30.77	31.40	31.40
JW	2.59	2.57	2.68	38.0	38.4	39.9
MN	2.34	2.35	2.25	35.85	36.28	34.00
VR	2.11	2.17	2.28	31.70	32.55	33.90
AVE	**2.62**	**2.70**	**2.78**	**35.23**	**36.48**	**37.50**
SE	**0.23**	**0.22**	**0.24**	**2.10**	**2.08**	**2.29**

Study 3: Lactate Threshold			
Subject	**Base**	**End**	**% Change**
CM			
CG	1.9	1.95	2.6
DIT	1.65	1.8	9.1
EV	1.35	1.55	14.8
JF	1.2	1.65	37.5
MF	1.26	1.6	27.0
MP	1.25	1.28	2.4
NG	1.65	2	21.2
PB	1.25	1.55	24.0
PD	3	3.33	11.0
VE	1.55	1.8	16.1
YL	1.7	1.65	-2.9
AVG	**1.61**	**1.83**	**14.80**
SEM	**0.16**	**0.16**	**3.64**
CHO			
CF	1.83	1.95	6.6
JQ	1.05	1.25	19.0
KH	1.15	1.55	34.8
LM	1.4	1.85	32.1
MM	1.5	1.9	26.7
NF	1.6	1.6	0.0
RC	1.84	1.86	1.1
RM	1.95	2.2	12.8
TR	1.15	1.15	0.0
GM	1.25	1.35	8.0
AVG	**1.47**	**1.67**	**14.11**
SEM	**0.10**	**0.11**	**4.21**
PLA			
AR	0.95	1	5.3
BM	1.55	1.6	3.2
DT	1.7	2.1	23.5
JH	1.8	2.5	38.9
JL	1.25	1.45	16.0
JM	2.05	1.85	-9.8
JO	1.6	1.78	11.3
JW	1.65	1.72	4.2
MN	1.7	1.75	2.9
VR	1	1.25	25.0
AVG	**1.53**	**1.70**	**12.06**
SEM	**0.11**	**0.13**	**4.45**

	Study 3: Time Trial Performance Data					
CM	**TT Time (min)**			**TT Average Watts (W)**		
	TT2	**TT3**	**TT4**	**TT2**	**TT3**	**TT4**
CG	52.61	49.07	46.61	138	170	185
DIT	55.18	55.29	54.71	125	121	121
EV	54.33	51.13	51.14	119	132	134
JF	58.26	58.85	56.74	112	110	119
MF	52.11	54.78	53.09	142	123	130
MP	58.30	61.28	61.97	123	103	101
NG	46.43	45.69	45.12	188	195	202
PB	57.41	54.6	53.23	112	126	132
PD	43.38	42.68	42.36	254	269	271
VE	54.41	52.37	54.31	132	141	129
YL	54.44	51.07	50.8	129	148	154
AVE	**53.35**	**52.44**	**51.83**	**143.09**	**148.91**	**152.55**
SE	**1.42**	**1.63**	**1.67**	**12.76**	**14.44**	**14.81**
CHO	**TT Time (min)**			**TT Average Watts (W)**		
	TT2	**TT3**	**TT4**	**TT2**	**TT3**	**TT4**
CF	45.16	43.47	42.3	212	233	248
GM		58.99	64.05		107	93
JQ	55.04	56.13	53.78	111	107	116
KH	64.2	57.49	53.61	92	114	133
LM	49.67	48.86	48.24	153	159	164
MM	50.33	49.96	46.33	164	169	202
NF	48.26	47.30	48.82	161	167	158
RC	52.47	51.55	53.45	144	152	139
RM	62.48	55.91	48.71	100	121	166
TR	65.11	58.67	52.08	85	104	133
AVE	**54.75**	**52.83**	**51.14**	**135.78**	**143.30**	**155.20**
SE	**2.48**	**1.69**	**1.85**	**13.94**	**12.96**	**14.01**
PLA	**TT Time (min)**			**TT Average Watts (W)**		
	TT2	**TT3**	**TT4**	**TT2**	**TT3**	**TT4**
AR	81.51	85.02	73.97	69	71	76
BM	52.66	51.74	53.94	142	146	137
DT	56.06	52.66	50.68	144	164	173
JH	44.08	44.22	43.44	214	213	224
JL	63.71	55.51	54.26	93	118	122
JM	49.15	47.6	46.36	184	196	205
JO	56.45	52.68	52.42	123	144	139
JW	61.00	52.00	48.84	97	134	155
MN	61.33	58.63	61.33	97	107	97
VR	64.64	63.24	61.32	102	96	104
AVE	**59.06**	**56.33**	**54.66**	**126.50**	**138.90**	**143.20**
SE	**3.24**	**3.60**	**2.81**	**14.26**	**13.90**	**14.93**

Study 4

Subject	Treatment	Train Group	Age (yr)	Height (cm)	Baseline Weight (kg)	Base VO_2max (L/min)	Base VO_2max (ml/kg/min)	Gender
ALL SUBJECTS								
AVG			**22.0**	**168.6**	**71.7**	**27.5**	**11.0**	M=16
SEM			**0.5**	**1.5**	**2.4**	**3.0**	**2.4**	F=16
CM								
CG	1	4	19	176.0	75.96	41.5	3.17	M
DIT	1	3	19.3	166.1	55.75	2.15	38.00	F
EV	1	4	20.8	159.3	62.4	37.8	2.42	F
JF	1	2	22.8	172.0	67	38.90	2.63	M
MF	1	3	26.1	157.5	51.1	1.86	35.35	F
MP	1	3	24.5	162.6	63	1.63	25.40	F
NG	1	4	20.7	168.1	81.3	42.0	3.48	M
PB	1	2	22.2	179.6	64.2	35.35	2.32	M
PD	1	1	24.4	181.1	113.9	41.70	4.78	M
VE	1	3	22.9	168.4	74.4	2.54	33.96	F
YL	1	1	20	169.7	70.8	35.45	2.55	M
AVG			**22.1**	**169.1**	**70.9**	**25.5**	**14.0**	
SEM			**0.7**	**2.3**	**5.1**	**5.7**	**4.7**	
CHO								
CF	2	1	19.4	178.6	81.34	47.70	3.90	M
GM	2	2	20.2	157.5	67.5	28.60	1.97	F
JQ	2	4	22.8	149.9	51.9	34.5	1.81	F
KH	2	2	20.6	171.5	67.1	27.05	1.84	M
LM	2	4	29.2	163.3	65.4	36.2	2.41	F
MM	2	2	22.8	170.2	84	39.80	3.40	M
NF	2	4	18.8	175.3	60.53	46.4	2.85	M
RC	2	1	18.9	178.6	83.6	38.05	3.21	M
RM	2	4	22.8	168.1	80.4	37.9	3.04	M
SB	2	3	20.6	163.8	69.7	2.04	28.70	F
TR	2	4	18.3	171.7	71.76	27.9	2.04	F
AVG			**21.3**	**168.0**	**71.2**	**33.3**	**5.0**	
SEM			**0.9**	**2.7**	**3.1**	**3.7**	**2.4**	
PLA								
AR	3	3	21.4	150.1	58.2	1.70	28.90	F
BM	3	3	19.4	169.7	70.31	2.50	34.65	F
DT	3	2	22.1	180.3	99.1	33.30	3.30	M
JH	3	4	20	175.8	73.8	52.3	3.85	M
JL	3	3	28.5	165.1	61.3	1.91	30.65	F
JM	3	2	26.3	180.3	97.9	36.20	3.57	M
JO	3	1	24.9	164.6	75.6	30.77	2.35	F
JW	3	4	22.8	177.3	66	38.0	2.59	M
MN	3	1	18.8	158.2	64.4	35.85	2.34	F
VR	3	3	21.3	166.4	65.4	2.11	31.70	F
AVG			**22.6**	**168.8**	**73.2**	**23.5**	**14.4**	
SEM			**1.0**	**3.1**	**4.5**	**6.1**	**4.7**	
Treatments: 1=CM; 2=CHO; 3=PLA								

Study 4	Glucose (mmol/L)											
CM												
Subject	Base	Mid	End		TT1 pre	TT1 post	TT2 pre	TT2 post	TT3 pre	TT3 post	TT4 pre	TT4 post
CG	3.91	3.77	3.75		3.53	3.21	3.79	3.41	3.68	3.78	3.89	4.93
DIT	3.99	4.33	4.05		4.30	5.23	3.91	4.42	3.48	3.82	3.74	3.19
EV	3.49	3.82	3.83		3.83	3.55	3.84	4.19	3.65	4.52	3.85	4.67
JF	4.65	4.12	4.67		4.58	4.26	4.19	3.60	4.32	4.13	4.57	4.22
MF	4.24	4.44	4.20		4.22	4.79	4.24	4.72	4.22	4.58	4.24	4.70
MP	4.29	3.68	4.39		4.10	4.14	3.88	4.06	4.20	4.00	4.50	3.55
NG	4.52	4.36	4.32		4.30	4.59	4.49	4.78	4.82	4.61	4.74	5.08
PB	4.27	3.69	4.01		3.65	3.68	4.63	3.29	3.97	3.34	3.82	3.52
PD	4.61	4.57	4.47		4.71	5.37	4.56	5.00	4.54	5.00	4.46	5.41
VE	4.31	4.66	3.80		4.14	4.75	3.68	3.78	4.02	4.15	4.30	3.93
YL	5.22	4.97	4.75		5.23	4.36	4.67	4.37	4.86	4.54	4.79	4.53
AVG	**4.32**	**4.22**	**4.20**		**4.23**	**4.36**	**4.17**	**4.15**	**4.16**	**4.22**	**4.26**	**4.34**
SEM	**0.14**	**0.13**	**0.10**		**0.15**	**0.21**	**0.11**	**0.17**	**0.14**	**0.14**	**0.12**	**0.22**
CHO												
	Base	Mid	End		TT1 pre	TT1 post	TT2 pre	TT2 post	TT3 pre	TT3 post	TT4 pre	TT4 post
CF	4.27	3.92	4.09		4.08	5.51	3.86	4.91	4.35	5.21	3.97	6.52
JQ	5.20	5.28	5.06		5.08	5.58	4.94	5.24	5.22	5.65	4.92	5.22
KH	4.46	4.03	4.17		3.84	3.64	3.82	3.62	4.08	3.80	3.82	3.97
LM	4.06	3.46	3.93		4.01	5.19	4.03	5.21	4.29	4.51	4.07	4.47
MM	5.20	4.57	5.01		4.65	4.42	4.52	4.57	5.32	4.51	5.30	4.98
NF	3.78	3.94	3.78		3.86	4.00	3.58	3.39	3.98	4.18	3.90	4.70
RC	4.60	4.32	3.77		4.32	4.75	4.09	4.43	4.12	4.48	3.82	3.63
RM	3.71	4.06	4.72		3.95	3.47	3.85	3.53	3.92	3.73	3.85	3.71
SB	3.47	3.89	3.90		3.67	3.67	4.30	3.93	3.87	3.36	3.88	3.58
TR	3.21	3.88	3.59		3.41	3.30	3.49	3.34	3.47	3.32	3.53	3.47
AVG	**4.20**	**4.13**	**4.20**		**4.09**	**4.35**	**4.05**	**4.22**	**4.26**	**4.27**	**4.11**	**4.42**
SEM	**0.22**	**0.16**	**0.17**		**0.15**	**0.27**	**0.14**	**0.24**	**0.18**	**0.24**	**0.18**	**0.31**
PLA												
	Base	Mid	End		TT1 pre	TT1 post	TT2 pre	TT2 post	TT3 pre	TT3 post	TT4 pre	TT4 post
AR	4.09	4.17	4.82		4.36	3.23	4.23	3.49	4.22	3.84	4.38	3.65
BM	4.88	4.61	4.54		4.37	3.60	4.58	3.75	4.40	3.65	4.50	4.03
JH	4.85	4.58	4.57		4.15	4.04	4.65	4.23	4.96	4.33	5.00	5.14
JL	4.61	4.53	5.09		4.37	5.93	4.44	5.40	5.12	5.46	4.49	5.62
JM	4.33	4.39	4.54		4.16	3.98	4.38	3.96	4.35	3.95	4.47	5.08
JO	4.96	4.35	4.39		4.16	5.00	3.91	5.13	4.37	5.14	4.12	5.05
JW	4.03	3.68	3.88		4.05	4.00	3.91	4.01	4.25	4.53	4.20	4.12
MN	4.50	5.02	5.87		5.65	5.08	5.26	5.03	4.81	5.45	5.22	4.93
VR	4.69	4.24	4.27		4.22	3.62	4.31	4.19	4.02	3.81	4.61	4.14
AVG	**4.55**	**4.40**	**4.66**		**4.39**	**4.28**	**4.41**	**4.35**	**4.50**	**4.46**	**4.55**	**4.64**
SEM	**0.11**	**0.12**	**0.19**		**0.16**	**0.29**	**0.14**	**0.22**	**0.12**	**0.24**	**0.12**	**0.22**

Study 4	Insulin (pmol/L)		
	CM		
Subject	Base	Mid	End
CG	151.26	166.75	121.40
DIT	127.27	87.33	112.55
EV	106.40	127.65	133.41
JF	126.19	106.40	113.06
MF	53.40	67.90	80.73
MP	75.80	101.19	84.22
NG	112.75	156.07	120.12
PB	101.21	73.49	89.07
PD	89.87	93.90	118.20
VE	61.53	60.28	59.17
YL	124.25	104.24	81.88
AVE	**102.72**	**104.11**	**101.26**
SE	**9.10**	**10.33**	**6.98**
	CHO		
Subject	Base	Mid	End
CF	69.24	71.46	69.66
JQ	127.02	78.76	110.29
KH	151.75	118.41	173.07
LM	84.52	54.73	54.73
MM	95.77	117.86	110.77
NF	184.72	229.26	107.67
RC	132.44	152.72	220.43
RM	97.30	66.46	89.80
SB	83.34	120.50	111.88
TR	171.65	143.55	185.64
AVE	**119.78**	**115.37**	**123.39**
SE	**12.63**	**16.51**	**16.73**

	PLA		
Subject	Base	Mid	End
AR	205.35	247.88	353.97
BM	110.77	148.13	101.42
JH	97.34	70.55	101.80
JL	86.92	89.63	121.33
JM	127.58	116.05	80.08
JO	114.45	72.78	136.89
JW	83.55	63.62	64.31
MN	165.08	98.76	110.56
VR	158.69	102.65	95.42
AVE	**127.75**	**112.23**	**129.53**
SE	**13.63**	**19.06**	**28.93**

Study 4	**Glucose/Insulin Ratio**		
CM	Base	Mid	End
CG	3.2	2.8	3.9
DIT	3.9	6.2	4.5
EV	4.1	3.7	3.6
JF	4.6	4.8	5.2
MF	9.9	8.2	6.5
MP	7.1	4.5	6.5
NG	5.0	3.5	4.5
PB	5.3	6.3	5.6
PD	6.4	6.1	4.7
VE	8.8	9.7	8.0
YL	5.3	6.0	7.3
AVG	**5.8**	**5.6**	**5.5**
SEM	**0.6**	**0.6**	**0.4**
CHO	Base	Mid	End
CF	7.7	6.9	7.3
JQ	5.1	8.4	5.7
KH	3.7	4.3	3.0
LM	6.0	7.9	9.0
MM	6.8	4.9	5.7
NF	2.6	2.1	4.4
RC	4.3	3.5	2.1
RM	4.8	7.6	6.6
SB	5.2	4.0	4.4
TR	2.3	3.4	2.4
AVG	**4.8**	**5.3**	**5.1**
SEM	**0.5**	**0.7**	**0.7**
PLA	Base	Mid	End
AR	2.5	2.1	1.7
BM	5.5	3.9	5.6
JH	6.2	8.1	5.6
JL	6.6	6.3	5.2
JM	4.2	4.7	7.1
JO	5.4	7.5	4.0
JW	6.0	7.2	7.5
MN	3.4	6.4	6.6
VR	3.7	5.2	5.6
AVG	**4.8**	**5.7**	**5.4**
SEM	**0.5**	**0.6**	**0.6**

Study 4	Lactate (mmol/L)							
	CM							
	TT1_pre	TT1_post	TT2_pre	TT2_post	TT3_pre	TT3_post	TT4_pre	TT4_post
CG	0.52	2.73	0.51	5.46	0.79	4.84	0.80	8.16
DIT	1.05	6.47	0.83	2.87	1.06	2.05	1.07	2.19
EV	0.86	8.24	0.77	9.68	0.99	6.74	0.86	6.26
JF	0.96	7.42	1.09	4.25	0.68	1.69	1.42	2.26
MF	0.52	5.85	0.79	5.09	0.66	5.71	0.79	6.97
MP	1.07	10.29	1.06	8.22	1.10	7.00	1.24	6.06
NG	0.68	9.87	0.67	9.89	0.66	7.60	0.75	10.18
PB	0.80	6.27	1.00	3.92	0.71	4.05	0.70	3.43
PD	0.78	5.11	0.88	5.00	0.72	4.94	0.72	5.97
VE	0.54	4.09	0.68	1.84	0.55	3.16	0.95	1.63
YL	0.67	5.27	1.37	4.64	0.66	3.76	0.65	3.54
AVE	**0.77**	**6.51**	**0.88**	**5.53**	**0.78**	**4.69**	**0.90**	**5.15**
SE	**0.06**	**0.70**	**0.07**	**0.80**	**0.06**	**0.59**	**0.07**	**0.83**
	CHO							
Subject	TT1_pre	TT1_post	TT2_pre	TT2_post	TT3_pre	TT3_post	TT4_pre	TT4_post
CF	2.05	8.88	2.70	8.40	1.57	8.81	0.58	10.73
JQ	0.64	9.51	0.86	7.49	0.84	6.46	0.77	6.75
KH	0.64	2.80	0.84	4.20	0.80	3.33	0.72	4.78
LM	1.21	5.53	0.84	6.90	0.99	4.22	0.78	6.39
MM	0.63	4.26	0.67	5.97	0.69	3.53	0.77	6.59
NF	1.60	7.60	0.80	3.66	0.82	4.77	0.72	5.88
RC	0.91	4.69	1.26	3.93	0.69	3.58	0.69	2.41
RM	0.57	4.28	0.80	3.02	0.51	2.83	0.99	9.78
SB	0.98	5.75	0.58	0.52	0.71	5.58	0.84	6.92
TR	0.56	3.40	0.63	3.50	0.64	3.46	0.99	3.46
AVE	**0.98**	**5.67**	**1.00**	**4.76**	**0.83**	**4.66**	**0.78**	**6.37**
SE	**0.16**	**0.72**	**0.20**	**0.76**	**0.09**	**0.58**	**0.04**	**0.80**
	PLA							
	TT1_pre	TT1_post	TT2_pre	TT2_post	TT3_pre	TT3_post	TT4_pre	TT4_post
AR	1.14	6.20	1.11	5.54	0.82	3.28	1.27	2.26
BM	0.73	4.00	1.02	4.96	0.90	4.00	0.80	3.39
JH	0.76	6.47	0.64	7.03	0.96	5.82	0.49	7.49
JL	1.02	5.93	0.58	4.07	0.64	4.70	0.57	3.92
JM	0.93	3.24	0.80	7.55	0.81	6.68	0.66	7.58
JO	1.02	4.02	0.80	3.64	0.76	2.59	0.67	2.68
JW	0.80	2.50	0.62	1.11	0.68	2.22	0.89	3.13
MN	2.38	2.42	0.54	1.80	0.86	3.20	0.61	1.75
VR	0.66	2.47	2.80	3.24	0.79	2.80	1.06	2.38
AVE	**1.05**	**4.14**	**0.99**	**4.33**	**0.80**	**3.92**	**0.78**	**3.84**
SE	**0.17**	**0.55**	**0.24**	**0.73**	**0.03**	**0.51**	**0.08**	**0.73**

Study 4	Cortisol (pmol/L)							
	CM							
	Base	Mid	End		TT2_pre	TT2_post	TT4_pre	TT4_post
CG	992.5	1104.8	942.8		1064.6	1088.9	707.2	1340.5
DIT	1887.5	2554.5	2237.8		2170.3	2290.2	2514.3	2178.1
EV	2259.2	2038.6	2156.5		2538.8	2579.4	2268.4	2361.8
JF	756.6	847.5	658.7		340.2	320.2	855.9	460.7
MF	824.2	570.2	615.6		634.6	986.8	633.9	1275.0
MP	776.2	2613.1	1621.6		1732.8	2001.1	2712.5	2093.0
NG	694.7	869.9	813.7		986.1	1312.2	915.6	1582.3
PB	741.9	898.7	738.3		769.0	779.8	798.5	621.3
PD	945.0	725.6	723.9		965.6	891.8	895.4	843.8
VE	705.4	1001.3	766.0		703.1	900.7	569.4	723.8
YL	940.3	681.3	631.5		608.6	1027.7	729.0	924.4
AVE	**1047.6**	**1264.1**	**1082.4**		**1137.6**	**1289.0**	**1236.4**	**1309.5**
SE	**157.9**	**228.7**	**186.3**		**211.8**	**210.6**	**248.2**	**201.2**
	CHO							
Subject	Base	Mid	End		TT2_pre	TT2_post	TT4_pre	TT4_post
CF	874.3	814.6	870.0		827.0	1009.9	799.1	1060.4
JQ	1159.3	913.5	1158.8		838.0	1157.5	391.5	1207.6
KH	1119.7	753.8	577.1		779.6	772.5	818.2	866.3
LM	983.4	1074.2	1111.9		1026.9	997.1	1021.8	930.2
MM	782.9	702.1	758.9		755.3	630.3	892.9	716.5
NF	1268.2	1042.0	872.5		815.6	880.5	812.4	1127.2
RC	792.8	901.1	648.6		805.8	908.6	778.3	585.9
RM	639.9	584.5	606.6		657.3	470.1	598.8	640.7
SB	1048.3	931.5	843.2		1162.8	802.6	863.0	1177.7
TR	864.6	886.1	802.8		765.8	717.3	687.3	723.1
AVE	**953.3**	**860.3**	**825.0**		**843.4**	**834.7**	**766.4**	**903.6**
SE	**62.0**	**47.5**	**61.8**		**46.0**	**63.4**	**54.9**	**73.2**
	PLA							
	Base	Mid	End		TT2_pre	TT2_post	TT4_pre	TT4_post
AR	855.1	904.4	652.6		708.2	641.6	765.2	455.5
BM	592.2	650.8	796.9		689.8	782.9	828.8	617.9
JH	866.7	810.9	1061.4		920.8	1213.3	1076.3	1033.6
JL	548.5	1194.5	1111.7		1134.2	1520.0	1004.8	934.7
JM	986.8	724.0	717.6		781.0	823.3	940.4	848.4
JO	392.6	564.4	647.2		565.4	551.5	502.5	498.2
JW	1107.9	994.5	740.8		944.1	530.6	818.7	918.3
MN	757.4	772.0	833.8		763.8	768.2	805.3	785.8
VR	1432.8	1573.0	1520.3		1343.8	1147.3	1766.1	1683.3
AVE	**837.8**	**909.8**	**898.0**		**872.3**	**886.5**	**945.3**	**864.0**
SE	**105.3**	**103.9**	**95.3**		**81.0**	**112.0**	**116.2**	**122.2**

Study 4	IL-6 (pg/mL)							
	CM							
	Base	Mid	End		TT2_pre	TT2_post	TT4_pre	TT4_post
CG	0	0	0		0	0	0	12
DIT	2.455	0	0		0	0	0.24	7.795
EV	0	0	0		0	0	0	9.335
JF	0	0	0		0	0	0	0
MF	6.525	10.615	9.65		14.695	19.035	5.385	19.41
MP	1.115	0	1.725		0	0	0	3.305
NG	18.305	19.625	19.83		20.02	45.15	27.405	32.685
PB	0.28	0	0.15		0	1.49	0.28	0.815
PD	0	0	0		0	0	0	0.495
VE	0	0	0		0	0	0	0
YL	8.23	7.44	7.85		7.24	8.725	7.235	8.965
AVE	**3.36**	**3.43**	**3.56**		**3.81**	**6.76**	**3.69**	**8.62**
SE	**1.73**	**1.96**	**1.93**		**2.15**	**4.24**	**2.49**	**3.03**

	CHO							
Subject	Base	Mid	End		TT2_pre	TT2_post	TT4_pre	TT4_post
CF	54.14	18.16	55.09		51.16	77.255	32.38	23.225
JQ	0	0.15	0		0	11.2	0	7.995
KH	3.9	3.09	1.785		1.79	3.8	2.26	4.385
LM	0	0	0		0	0	0	1.57
NF	3.58	0.28	0.655		0.56	0.875	0.775	3.165
RC	6.875	0	10.065		8.86	24.87	13.81	17.065
RM	2.06	1.485	3.745		0	0	0.74	2.81
SB	3.5	3.635	5.35		4.05	9.29	8.7	6.315
TR	0	0	0		0	1.3	2.03	3.305
AVE	**8.23**	**2.98**	**8.52**		**7.38**	**14.29**	**6.74**	**7.76**
SE	**5.79**	**1.95**	**5.93**		**5.56**	**8.32**	**3.57**	**2.48**
	PLA							
	Base	Mid	End		TT2_pre	TT2_post	TT4_pre	TT4_post
AR	24.57	74.055	56.705		50.345	65.35	69.37	100.24
BM	11.76	11.435	45.015		7.165	16.095	11	41.035
JH	6.635	7.905	3.24		3.16	7.265	5.115	26.04
JL	0.54	2.065	0.93		0.185	4.93	0	3.395
JM	0	1.61	0.895		1.46	0	0	3.215
JO	2.01	2.04	0.21		0.255	1.62	0.525	0.13
JW	16.205	14.215	11.28		13.995	14.57	10.47	22.95
MN	0	0	0		0	0	0	0
VR	38.68	29.315	36.755		28.97	39.295	38.33	43.3
AVE	**11.16**	**15.85**	**17.23**		**11.73**	**16.57**	**14.98**	**26.70**
SE	**4.46**	**7.90**	**7.51**		**5.77**	**7.35**	**7.92**	**10.81**

Study 4	IL-8 (pg/mL)							
	CM							
	Base	Mid	End		TT2_pre	TT2_post	TT4_pre	TT4_post
CG	0.495	1.58	2.065		0.88	3.615	2.875	8.99
DIT	2.18	1.7	1.185		1.5	2.985	1.785	2.305
EV	4.465	3.165	1.98		0.58	5.245	3.495	5.26
JF	7.925	0	0.765		0	0	1.675	0.905
MF	8.23	10.56	8.755		13.24	13.825	8.56	16.51
MP	5.755	9.385	8.12		2.335	6.06	2.385	6
NG	19.43	17.065	17.865		7.56	21.11	18.955	30.82
PB	4.455	5.435	5.975		2.98	4.465	4.59	4.89
PD	2.935	2.33	2.765		2.015	3.375	3.595	4.13
VE	9.275	4.345	3.37		4.365	5.045	4.39	4.875
YL	2.955	2.71	2.75		2.495	1.635	1.525	2.945
AVE	**6.19**	**5.30**	**5.05**		**3.45**	**6.12**	**4.89**	**7.97**
SE	**1.56**	**1.53**	**1.52**		**1.16**	**1.83**	**1.53**	**2.61**
	CHO							
Subject	Base	Mid	End		TT2_pre	TT2_post	TT4_pre	TT4_post
CF	26.82	9.91	22.195		24.69	34.29	20.03	47.085
JQ	0.645	0.955	1.77		0.62	1.845	0.815	1.935
KH	2.76	2.56	1.65		3.47	4.6	2.64	3.8
LM	2.675	0.86	0.54		1.6	1.92	0.97	1.965
NF	2.485	3.445	1.66		1.765	2.135	2.11	3.155
RC	6.665	3.305	24.08		11.53	21.525	17.885	27.615
RM	11.53	7.1	5.26		3.965	5.695	7.605	12.745
SB	85.595	60.32	59.31		70.395	95.3	55.12	72.155
TR	2.4	2.515	4.06		2.85	5	6.175	11.415
AVE	**15.73**	**10.11**	**13.39**		**13.43**	**19.15**	**12.59**	**20.21**
SE	**9.15**	**6.35**	**6.49**		**7.56**	**10.21**	**5.82**	**8.20**
	PLA							
	Base	Mid	End		TT2_pre	TT2_post	TT4_pre	TT4_post
AR	25.01	28.585	23.235		21.55	34.515	25.52	37.015
BM	83.62	58.55	166.77		71.055	86.89	74	98.65
JH	31.385	31.555	17.825		11.61	17.465	14.92	27.265
JL	6.55	5.385	8.27		4.695	8.77	5.765	7.01
JM	4.41	7.255	9.94		3.245	4.35	0.755	12.475
JO	8.12	3.95	5.435		4.43	8.325	5.415	7.495
JW	4.1	7.145	5.62		4.725	9.24	7.265	11.145
MN	2.77	4.12	3.41		3.96	4.29	4.02	4.905
VR	13.39	19.04	15.905		15.735	15.805	17.585	17.685
AVE	**19.93**	**18.40**	**28.49**		**15.67**	**21.07**	**17.25**	**24.85**
SE	**8.63**	**6.15**	**17.42**		**7.24**	**8.79**	**7.56**	**9.87**

Study 4	IL-10 (pg/mL)							
	CM							
	Base	Mid	End		TT2_pre	TT2_post	TT4_pre	TT4_post
CG	0	0	0		0	0	0	0
DIT	4.68	1.415	1.74		1.605	7.22	1.415	5.76
EV	6.5	0	0		0	4.75	0	22.22
JF	0	0	0		0	0	0	0
MF	0.805	0.86	0.805		1.355	6.59	0.805	8.13
MP	0	0	0		0	1.05	0	5.685
NG	23.805	35.875	34.625		41.435	58.415	42.505	46.015
PB	3.73	4.105	2.985		2.315	7.825	1.79	4.215
PD	1.27	1.18	2.19		3.275	8.455	3.88	7.79
VE	0.501	0.92	1.835		0	0.51	0.825	0.19
YL	8.065	6.965	7.665		5.9	7.035	4.13	9.655
AVE	**4.49**	**4.67**	**4.71**		**5.08**	**9.26**	**5.03**	**9.97**
SE	**2.11**	**3.19**	**3.07**		**3.68**	**5.02**	**3.77**	**4.07**
	CHO							
Subject	Base	Mid	End		TT2_pre	TT2_post	TT4_pre	TT4_post
CF	1.68	1.7	3.89		3.86	4.525	1.71	2.39
JQ	0.47	1.02	0.995		1.33	5.525	0.86	0.32
KH	2.735	1.1	0		2.42	2	0.965	0.74
LM	2.855	2.615	2.09		2.785	14.835	2.395	21.645
NF	1.125	0	0		0	0.055	0	0.48
RC	12.58	8.2	9.96		11.8	10.7	10.43	8.705
RM	15.335	0.015	3.66		0.245	0.98	1.05	1.37
SB	2.725	1.385	1.495		1.1	5.025	1.675	4.56
TR	0	0	0		0	0	0	0
AVE	**4.39**	**1.78**	**2.45**		**2.62**	**4.85**	**2.12**	**4.47**
SE	**1.85**	**0.85**	**1.06**		**1.23**	**1.68**	**1.07**	**2.34**

	PLA							
	Base	Mid	End		TT2_pre	TT2_post	TT4_pre	TT4_post
AR	2.01	4.36	5.64		2.855	3.335	0.935	2.58
BM	0.19	0	0.295		0	0.71	0	0.785
JH	0	0	0		0	13.075	0.055	2.865
JL	0	1.3	0.54		0.295	7.005	0.425	1.3
JM	2.178	11.29	2.63		0.91	2.03	0	1.875
JO	8.425	9.98	6.955		5.71	10.915	6.485	7.945
JW	4.46	2.31	1.93		3.665	6.84	3.48	15.19
MN	0	0	0		1.025	6.9	3.48	3.955
VR	72.24	63.32	68.685		67.4	68.45	72.52	72.675
AVE	**9.94**	**10.28**	**9.63**		**9.10**	**13.25**	**9.71**	**12.13**
SE	**7.84**	**6.78**	**7.43**		**7.32**	**7.03**	**7.89**	**7.72**

Study 4	IL-1Ra (pg/mL)							
	CM							
	Base	Mid	End		TT2_pre	TT2_post	TT4_pre	TT4_post
CG	0	0	0		0	0	0	0
DIT	1.15	0	1.315		0	0.375	0	0
EV	0	58.2	0		0	50.815	0	34.335
JF	0	0	0		0	0	0	0
MF	0	0.44	1.26		3.69	1.75	0	2.135
MP	0	0	0		0	0	0	0
NG	7.285	18.84	23.345		19.06	48.01	32.335	35.02
PB	0	0	0.13		0	0	0	0
PD	0	0	0		0	0	0.78	13.81
VE	0	0	2.855		0	0	0	1.135
YL	6.145	3.86	6.72		5.575	1.355	3.285	8.14
AVE	**1.33**	**7.39**	**3.24**		**2.58**	**9.30**	**3.31**	**8.60**
SE	**0.81**	**5.36**	**2.10**		**1.75**	**5.99**	**2.92**	**4.11**

	CHO							
Subject	Base	Mid	End		TT2_pre	TT2_post	TT4_pre	TT4_post
CF	0.2	1.93	1.355		2.135	0.78	0	0.78
JQ	0	0	0		0	0	0	0
KH	0	0	0		1.825	0	6.21	0
LM	3	0	0		0	0.2	0	0.2
NF	2.91	0.13	0		0.13	1.65	0	0
RC	5.005	0.78	11.265		0	2.505	0	14.38
RM	0	0	0		0	0	0	0
SB	0	1.26	1.65		0.815	6.46	2.325	1.56
TR	4.46	12.99	0		0	0	0	0
AVE	**1.73**	**1.90**	**1.59**		**0.55**	**1.29**	**0.95**	**1.88**
SE	**0.70**	**1.41**	**1.23**		**0.29**	**0.71**	**0.71**	**1.57**
	PLA							
	Base	Mid	End		TT2_pre	TT2_post	TT4_pre	TT4_post
AR	0	0	0		0	0	0	0
BM	0	0	0		0	0	0	0
JH	0	0	0		0	0.805	0	4.44
JL	0	0	0		0	0	0	0
JM	0	0	0		0	0	0	0
JO	49.89	31.2	31.77		20.06	33.56	17.995	44.195
JW	0	0	0		0	0	2.295	0
MN	0	0	0		0	0	0	0
VR	39.065	36.37	43.535		32.335	45.775	45.775	47.565
AVE	**9.88**	**7.51**	**8.37**		**5.82**	**8.90**	**7.34**	**10.69**
SE	**6.60**	**4.98**	**5.62**		**3.98**	**5.90**	**5.19**	**6.67**

Study 4	IL-1B (pg/mL)							
	CM							
	Base	Mid	End		TT2_pre	TT2_post	TT4_pre	TT4_post
CG	0.00	0.00	0.00		0.00	0.00	0.00	17.62
DIT	4.00	0.39	0.67		2.14	4.22	0.50	1.38
EV	0.00	0.00	0.00		0.00	0.00	0.00	0.00
JF	0.00	0.00	0.00		0.00	0.00	0.00	0.00
MF	8.08	17.47	19.36		18.35	15.30	13.61	34.91
MP	2.47	1.24	0.09		2.09	4.67	1.01	2.40
NG	1.58	3.10	3.59		3.33	7.08	4.89	3.02
PB	1.44	0.53	0.00		2.19	0.29	0.00	0.34
PD	1.90	0.00	2.17		2.00	1.05	6.55	14.02
VE	0.29	0.14	1.38		0.00	0.00	0.04	1.93
YL	2.94	1.58	3.24		1.49	2.00	0.34	5.57
AVE	**2.06**	**2.22**	**2.77**		**2.87**	**3.15**	**2.45**	**7.38**
SE	**0.72**	**1.55**	**1.71**		**1.59**	**1.41**	**1.30**	**3.27**
	CHO							
Subject	Base	Mid	End		TT2_pre	TT2_post	TT4_pre	TT4_post
CF	7.49	11.32	7.65		7.70	11.51	8.48	10.88
JQ	0.54	0.16	0.95		0.37	0.43	1.54	1.12
KH	2.23	0.96	3.42		6.15	4.00	6.44	2.45
LM	15.52	2.18	3.71		6.47	6.92	2.02	12.35
NF	13.94	5.58	1.29		4.34	2.36	1.82	1.12
RC	8.99	5.05	21.51		15.79	27.19	15.20	26.05
RM	3.29	0.86	0.62		0.55	1.24	1.36	2.34
SB	1.92	2.14	2.42		0.98	7.16	1.92	1.82
TR	50.45	56.79	14.75		6.80	21.00	1.40	18.81
AVE	**11.60**	**9.45**	**6.26**		**5.46**	**9.09**	**4.46**	**8.55**
SE	**5.17**	**6.03**	**2.42**		**1.61**	**3.10**	**1.59**	**3.03**

	PLA							
	Base	Mid	End		TT2_pre	TT2_post	TT4_pre	TT4_post
AR	21.87	11.67	17.06		7.12	11.13	7.97	7.96
BM	1.46	0.29	1.82		3.13	5.26	2.50	4.01
JH	1.92	1.02	0.65		0.85	5.01	2.09	3.97
JL	1.93	0.29	0.83		0.51	3.29	1.38	1.71
JM	0.88	0.53	0.00		1.78	1.84	0.05	1.13
JO	7.76	6.30	7.92		5.04	4.31	2.16	6.82
JW	9.74	14.80	24.85		19.62	14.80	27.54	26.67
MN	0.00	3.93	0.25		0.00	0.00	0.00	3.05
VR	9.44	7.78	9.44		9.36	10.78	10.36	10.24
AVE	**6.11**	**5.18**	**6.98**		**5.27**	**6.27**	**6.00**	**7.28**
SE	**2.35**	**1.79**	**2.95**		**2.08**	**1.63**	**2.94**	**2.62**

Study 4	TNFα (pg/mL)							
	CM							
	Base	Mid	End		TT2_pre	TT2_post	TT4_pre	TT4_post
CG	0	0	0		0	0	0	2.895
DIT	46.085	54.945	37.59		35.085	41.74	34.645	27.525
EV	9.475	12.685	9.285		7.685	11.63	11.875	12.6
JF	10.62	0	0.96		0	0.295	0.025	0.855
MF	8.245	21.93	10.2		21.15	22.53	7.745	20.215
MP	3.965	3.1	3.425		2.66	4.145	2.835	4.59
NG	14.075	16.335	17.575		15.96	23.66	21.18	22.42
PB	6.135	8.28	5.835		5.99	6.325	6.775	5
PD	5.785	5.3	6.88		6.04	7.25	9.945	13.53
VE	0.295	1.58	1.9		0.85	1.215	0.85	1.24
YL	4.425	2.57	2.775		2.53	2.69	1.86	3.01
AVE	**9.92**	**11.52**	**8.77**		**8.90**	**11.04**	**8.89**	**10.35**
SE	**3.83**	**4.85**	**3.26**		**3.31**	**3.96**	**3.22**	**2.86**
	CHO							
Subject	Base	Mid	End		TT2_pre	TT2_post	TT4_pre	TT4_post
CF	16.61	14.235	24.195		20.05	22.985	17.105	17.21
JQ	1.005	1.72	2.42		1.98	2.625	2.3	2.86
KH	6.77	6.605	7.77		9.59	8	10.48	7.49
LM	5.115	2.61	2.525		2.695	2.975	1.86	4.39
NF	6.585	4.09	2.78		4.87	5.1	2.765	2.935
RC	14.72	13.89	14.87		16.075	16.885	16	21.49
RM	4.29	6.19	6.215		3.965	4.115	6.06	7.455
SB	12.315	15.355	18.565		13.44	17.965	19.345	19.3
TR	8.42	10.71	5.935		5.425	6.2	5.385	6.105
AVE	**8.43**	**8.38**	**9.48**		**8.68**	**9.65**	**9.03**	**9.92**
SE	**1.71**	**1.76**	**2.63**		**2.16**	**2.53**	**2.30**	**2.45**

	PLA							
	Base	Mid	End		TT2_pre	TT2_post	TT4_pre	TT4_post
AR	5.535	2.86	6.725		4.2	3.07	4.02	3.16
BM	7.685	11.35	32.77		12.485	41.235	12	27.94
JH	11.16	10.435	6.925		19.62	8.22	7.03	20.11
JL	4.245	3.255	4.65		3.035	3.52	3.955	4.89
JM	6.905	16.365	12.45		5.1	6.97	4.86	7.21
JO	61.765	24.695	49.655		29.61	62.175	29.045	56.055
JW	8.395	8.31	12.16		10.63	9.215	14.835	15.86
MN	3.52	6.515	4.495		5.38	3.525	6.26	7.73
VR	15.925	13.395	12.895		13.5	13.665	16.005	14.465
AVE	**13.90**	**10.80**	**15.86**		**11.51**	**16.84**	**10.89**	**17.49**
SE	**6.11**	**2.28**	**5.11**		**2.89**	**6.91**	**2.74**	**5.50**

	Study 4	Body Composition Data														
Subject	Tmt Grp	Wt kg Base	Wt kg Mid	Wt kg End	LM% Base	LM% Mid	LM% End	LM (g) Base	LM (g) Mid	LM (g) End	FM% Base	FM% Mid	FM% End	FM (g) Base	FM (g) Mid	FM (g) End
NG	1	81.3	81.8	81.5	68.4	68.1	68.9	55605	55711	56152	27.4	27.6	26.9	22297	22554	21926
JF	1	67	67.6	67.7	80.9	81.8	82.5	54175	55325	55841	14.9	13.9	13.4	9997	9412	9050
PB	1	64.2	63.8	64.2	69.2	71.8	73.3	44430	45835	47036	26.5	24	22.5	17008	15289	14451
PD	1	113.9	114.6	113.8	66.5	67.3	67.9	75749	77112	77299	29	28.3	27.6	33066	32438	31448
YL	1	70.8	70.5	71.1	75.7	75.5	76.3	53565	53197	54243	20.6	20.9	20.1	14610	14703	14267
DIT	1	55.75	55.92	56.11	65.4	66.5	66.0	36470	37166	37032	30	29	29.4	16706	16220	16475
MF	1	51.1	51.2	50.3	63.1	64.9	65.8	32221	33227	33109	32.7	31	30	16691	15844	15071
VE	1	74.4	73.7	73.9	59.5	62.3	63.2	44299	45904	46741	36.3	33.6	32.7	27020	24771	24130
CG	1	75.96	73.84	74.26	79.0	80.5	82.0	60004	59478	60902	16.9	15.1	13.7	12802	11170	10154
EV	1	62.4	63	64.3	62.6	62.7	64.2	39034	39501	41275	33.1	32.8	31.5	20654	20690	20249
CF	2	81.34	81.6	83.1	87.5	85.9	85.6	71184	70072	71115	8.1	9.7	10.1	6565	7902	8371
GM	2	67.5	66.8	67.4	57.1	56.1	57.3	38555	37481	38610	39.2	40.1	39.1	26412	26778	26382
KH	2	67.1	68.8	70.6	76.1	77.8	77.0	51087	53547	54352	20	18.5	19.4	13457	12766	13667
MM	2	84	81.9	82.5	71.5	73.0	74.2	60099	59752	61181	24.4	23	21.7	20504	18823	17919
RC	2	83.6	82.2	82.8	68.4	66.7	68.6	57173	54839	56794	27.2	28.5	26.8	22739	23456	22225
RM	2	80.4	79	79.1	69.9	72.5	71.6	56165	57258	56646	25.9	23.3	24	20834	18398	19012
TR	2	71.76	72.78	72.45	58.1	58.2	59.2	41702	42367	42865	37.9	37.8	36.9	27230	27480	26754
JQ	2	51.9	51.3	51.5	63.3	64.7	65.5	32868	33194	33730	32.6	31.3	30.5	16937	16065	15704
LM	2	65.4	63.4	63.4	60.0	59.9	61.4	39237	37977	38953	36.5	36.4	34.9	23835	23063	22105
NF	2	60.53	61.15	61.39	76.3	75.6	75.5	46159	46219	46320	19.5	20.1	20.2	11797	12269	12414
JM	3	97.9	98.1	96.6	66.0	67.1	66.3	64587	65777	64043	30.3	29.3	30	29707	28769	28980
JO	3	75.6	74.9	74.9	61.4	61.6	63.1	46384	46164	47241	34.3	34	32.6	25,951	25442	24451
MN	3	64.4	64.7	65.4	60.3	60.5	60.5	38861	39129	39552	35.6	35.3	35.4	22901	22828	23170
AR	3	58.2	58.3	58.9	57.2	58.6	60.7	33298	34146	35774	38.9	37.7	35.8	22607	21976	21077
BM	3	70.31	68.78	67.47	58.1	58.8	60.0	40824	40420	40456	37.6	36.8	35.6	26450	25334	23989
JL	3	61.3	61	61.1	64.3	64.7	65.1	39390	39454	39760	32	31.6	31.3	19639	19263	19116
VR	3	65.4	65.6	66	57.1	58.8	59.6	37338	38581	39303	38.9	37.3	36.5	25421	24450	24101
JH	3	73.8	72.3	73.4	84.7	85.4	86.4	62495	61751	63445	11.4	10.6	9.6	8401	7633	7039
JW	3	66	66.5	66.4	80.4	80.6	81.4	53071	53601	54025	15.1	15	14.4	9949	9994	9536
DT	3	99.1	99.7	99.3	61.6	61.9	62.0	61055	61763	61546	34.7	34.2	34.1	34358	34082	33902
CM	**Avg**	**71.7**	**71.6**	**71.7**	**69.0**	**70.1**	**71.0**	**49555.2**	**50245.6**	**50963.0**	**26.7**	**25.6**	**24.8**	**19085.1**	**18309.1**	**17722.1**
	SE	**5.5**	**5.6**	**5.5**	**2.3**	**2.2**	**2.3**	**4090.1**	**4063.2**	**4074.0**	**2.3**	**2.2**	**2.2**	**2183.3**	**2180.1**	**2153.1**
CHO	**Avg**	**71.4**	**70.9**	**71.4**	**68.8**	**69.0**	**69.6**	**49422.9**	**49270.6**	**50056.6**	**27.1**	**26.9**	**26.4**	**19031.0**	**18700.0**	**18455.3**
	SE	**3.4**	**3.3**	**3.4**	**3.0**	**3.0**	**2.8**	**3744.6**	**3708.3**	**3743.2**	**3.1**	**3.1**	**2.9**	**2126.9**	**2066.2**	**1910.5**
PLA	**Avg**	**73.2**	**73.0**	**72.9**	**65.1**	**65.8**	**66.5**	**47730.3**	**48078.6**	**48514.5**	**30.9**	**30.2**	**29.5**	**22538.4**	**21977.1**	**21536.1**
	SE	**4.5**	**4.6**	**4.4**	**3.1**	**3.0**	**3.0**	**3685.2**	**3680.4**	**3551.1**	**3.1**	**3.0**	**3.0**	**2567.4**	**2537.5**	**2565.4**

Lean and Fat Mass Differential, Whole Body									
Subject	**Lean mass (g) Base**	**Lean mass (g) Mid**	**Lean mass (g) End**	**Lean Mass (g)Δ**	**Fat mass (g) Base**	**Fat mass (g) Mid**	**Fat mass (g) End**	**Fat Mass (g) Δ**	**DIFF (g)**
CM									
CG	60004.0	59478.0	60902.0	898.0	12802.0	11170.0	10154.0	-2648.0	3546.0
DIT	36470.0	37166.0	37032.0	562.0	16706.0	16220.0	16475.0	-231.0	793.0
EV	39034.0	39501.0	41275.0	2241.0	20654.0	20690.0	20249.0	-405.0	2646.0
JF	54175.0	55325.0	55841.0	1666.0	9997.0	9412.0	9050.0	-947.0	2613.0
MF	32221.0	33227.0	33109.0	888.0	16691.0	15844.0	15071.0	-1620.0	2508.0
NG	55605.0	55711.0	56152.0	547.0	22297.0	22554.0	21926.0	-371.0	918.0
PB	44430.0	45835.0	47036.0	2606.0	17008.0	15289.0	14451.0	-2557.0	5163.0
PD	75749.0	77112.0	77299.0	1550.0	33066.0	32438.0	31448.0	-1618.0	3168.0
VE	44299.0	45904.0	46741.0	2442.0	27020.0	24771.0	24130.0	-2890.0	5332.0
YL	53565.0	53197.0	54243.0	678.0	14610.0	14703.0	14267.0	-343.0	1021.0
AVG	**49555.2**	**50245.6**	**50963.0**	**1407.8**	**19085.1**	**18309.1**	**17722.1**	**-1363.0**	**2770.8**
SEM	**4090.1**	**4063.2**	**4074.0**	**254.0**	**2183.3**	**2180.1**	**2153.1**	**332.3**	**511.6**
CHO									
CF	71184.0	70072.0	71115.0	-69.0	6565.0	7902.0	8371.0	1806.0	-1875.0
GM	38555.0	37481.0	38610.0	55.0	26412.0	26778.0	26382.0	-30.0	85.0
JQ	32868.0	33194.0	33730.0	862.0	16937.0	16065.0	15704.0	-1233.0	2095.0
KH	51087.0	53547.0	54352.0	3265.0	13457.0	12766.0	13667.0	210.0	3055.0
LM	39237.0	37977.0	38953.0	-284.0	23835.0	23063.0	22105.0	-1730.0	1446.0
MM	60099.0	59752.0	61181.0	1082.0	20504.0	18823.0	17919.0	-2585.0	3667.0
NF	46159.0	46219.0	46320.0	161.0	11797.0	12269.0	12414.0	617.0	-456.0
RC	57173.0	54839.0	56794.0	-379.0	22739.0	23456.0	22225.0	-514.0	135.0
RM	56165.0	57258.0	56646.0	481.0	20834.0	18398.0	19012.0	-1822.0	2303.0
TR	41702.0	42367.0	42865.0	1163.0	27230.0	27480.0	26754.0	-476.0	1639.0
AVG	**49422.9**	**49270.6**	**50056.6**	**633.7**	**19031.0**	**18700.0**	**18455.3**	**-575.7**	**1209.4**
SEM	**3744.6**	**3708.3**	**3743.2**	**339.9**	**2126.9**	**2066.2**	**1910.5**	**413.4**	**541.3**

PLA									
AR	33298.0	34146.0	35774.0	2476.0	22607.0	21976.0	21077.0	-1530.0	4006.0
BM	40824.0	40420.0	40456.0	-368.0	26450.0	25334.0	23989.0	-2461.0	2093.0
DT	61055.0	61763.0	61546.0	491.0	34358.0	34082.0	33902.0	-456.0	947.0
JH	62495.0	61751.0	63445.0	950.0	8401.0	7633.0	7039.0	-1362.0	2312.0
JL	39390.0	39454.0	39760.0	370.0	19639.0	19263.0	19116.0	-523.0	893.0
JM	64587.0	65777.0	64043.0	-544.0	29707.0	28769.0	28980.0	-727.0	183.0
JO	46384.0	46164.0	47241.0	857.0	25951.0	25442.0	24451.0	-1500.0	2357.0
JW	53071.0	53601.0	54025.0	954.0	9949.0	9994.0	9536.0	-413.0	1367.0
MN	38861.0	39129.0	39552.0	691.0	22901.0	22828.0	23170.0	269.0	422.0
VR	37338.0	38581.0	39303.0	1965.0	25421.0	24450.0	24101.0	-1320.0	3285.0
AVG	**47730.3**	**48078.6**	**48514.5**	**784.2**	**22538.4**	**21977.1**	**21536.1**	**-1002.3**	**1786.5**
SEM	**3685.2**	**3680.4**	**3551.1**	**292.0**	**2567.4**	**2537.5**	**2565.4**	**246.1**	**394.1**

Lean and Fat Mass Differential, Legs													
Subj	**Reg Tiss Wt (g) Base**	**Reg Tiss Wt (g) Mid**	**Reg Tiss Wt (g) End**	**Total Reg Tiss Chg**	**FM (g) Base**	**FM (g) Mid**	**FM (g) End**	**Total FM (g) Chg**	**LM (g) Base**	**LM (g) Mid**	**LM (g) End**	**Total LM (g) Chg**	**DIFF (g)**
CM													
CG	25959	24763	24945	-1014	4436	3838	3680	-756	21523	20925	21266	-257	499
DIT	32281	32684	33571	1290	11137	11102	11177	40	21144	21582	22394	1250	1210
EV	18759	19251	19702	943	6554	6230	6333	-221	12205	13021	13369	1164	1385
JF	20302	20890	21129	827	3513	3312	3343	-170	16789	17579	17786	997	1167
MF	18256	17812	17670	-586	6925	6568	6228	-697	11332	11243	11442	110	807
NG	25032	25595	25052	20	5669	5862	5434	-235	19374	19733	19618	244	479
PB	20805	21173	20413	-392	5609	5251	4998	-611	15195	15922	15415	220	831
PD	35597	36499	35839	242	9976	10021	9645	-331	25620	26478	26194	574	905
VE	26477	26790	26664	187	11106	10578	10390	-716	15371	16212	16274	903	1619
YL	24599	23948	23451	-1148	4677	4634	4490	-187	19922	19314	18961	-961	-774
AVG	**24806.7**	**24940.5**	**24843.6**	**36.9**	**6960.2**	**6739.6**	**6571.8**	**-388.4**	**17847.5**	**18200.9**	**18271.9**	**424.4**	**812.8**
SEM	**1802.2**	**1862.5**	**1867.0**	**261.6**	**887.3**	**897.0**	**896.3**	**89.1**	**1414.2**	**1397.3**	**1390.8**	**220.2**	**210.7**
CHO													
CF	27505	28431	28345	840	2763	3301	3461	698	24742	25130	24884	142	-556
GM	24876	25395	25963	1087	10926	11616	11788	862	13950	13779	14176	226	-636
JQ	17116	17476	17164	48	6004	5981	5763	-241	11112	11495	11402	290	531
KH	24156	25243	26302	2146	6298	6354	6647	349	17858	18889	19655	1797	1448
LM	22454	22147	22174	-280	9347	9122	8937	-410	13107	13025	13237	130	540
MM	26922	26548	27155	233	6892	6569	6437	-455	20030	19979	20718	688	1143
NF	18427	18994	18740	313	3514	3675	3604	90	14913	15319	15136	223	133
RC	24597	26120	25886	1289	6084	6575	6181	97	18512	19546	19704	1192	1095
RM	25289	26057	23986	-1303	6725	6576	5835	-890	18563	19480	18150	-413	477
TR	22868	23537	23590	722	8877	9132	9301	424	13990	14405	14289	299	-125
AVG	**23421.0**	**23994.8**	**23930.5**	**509.5**	**6743.0**	**6890.1**	**6795.4**	**52.4**	**16677.7**	**17104.7**	**17135.1**	**457.4**	**405.0**
SEM	**1066.7**	**1103.6**	**1151.8**	**298.3**	**790.9**	**795.6**	**813.8**	**174.7**	**1274.0**	**1321.2**	**1315.6**	**197.6**	**223.9**
PLA													
AR	21334	21301	21551	217	9104	9126	8967	-137	12230	12174	12584	354	491
BM	24977	24243	23587	-1390	10419	9934	9484	-935	14558	14309	14103	-455	480

DT	18601	19616	18653	52	6506	6651	6171	-335	12095	12965	12482	387	722
JH	23829	23432	24156	327	3353	3096	3068	-285	20476	20336	21088	612	897
JL	21128	20184	20892	-236	7841	7328	7607	-234	13287	12857	13286	-1	233
JM	30323	31192	30773	450	8031	8196	8232	201	22929	22996	22541	-388	-589
JO	28424	28671	28012	-412	10998	10818	10427	-571	17426	17852	17585	159	730
JW	21143	21280	21261	118	3359	3291	3088	-271	17785	17988	18173	388	659
MN	21767	23758	23253	1486	8276	8966	8656	380	13491	14791	14597	1106	726
VR	21476	22221	22484	1008	9341	9454	9170	-171	12135	12767	13314	1179	1350
AVG	**23300.2**	**23589.8**	**23462.2**	**162.0**	**7722.8**	**7686.0**	**7487.0**	**-235.8**	**15641.2**	**15903.5**	**15975.3**	**334.1**	**569.9**
SEM	**1153.1**	**1173.5**	**1124.9**	**247.0**	**833.7**	**840.3**	**817.9**	**115.4**	**1211.6**	**1171.6**	**1155.1**	**172.6**	**158.7**

Lean and Fat Mass Differential, Trunk													
Subj	**Reg Tiss Wt (g) Base**	**Reg Tiss Wt (g) Mid**	**Reg Tiss Wt (g) End**	**Total Reg Tiss Chg (g)**	**FM (g) Base**	**FM (g) Mid**	**FM (g) End**	**Total FM (g) Chg**	**LM (g) Base**	**LM (g) Mid**	**LM (g) End**	**Total LM (g) Chg**	**DIFF (g)**
CM													
CG	33568	32879	33001	-567	7052	6220	5428	-1624	26516	26658	27573	1057	2681
DIT	46753	46574	45674	-1079	19312	19141	18849	-463	27442	27433	26826	-616	-153
EV	30633	30502	31256	623	11455	11730	11228	-227	19178	18772	20028	850	1077
JF	30593	30643	30972	379	5392	5081	4767	-625	25201	25562	26205	1004	1629
MF	22462	23223	22557	95	8049	7676	7328	-721	14413	15547	15230	817	1538
NG	39759	39523	40272	513	14407	14520	14322	-85	25352	25004	25951	599	684
PB	28915	28389	29724	809	9293	8204	7785	-1508	19622	20185	21939	2317	3825
PD	54976	54444	53667	-1309	20105	19431	18594	-1511	34870	35013	35074	204	1715
VE	33299	32718	33216	-83	12826	11374	11112	-1714	20473	21344	22104	1631	3345
YL	31833	32050	32808	975	8207	8217	8049	-158	23626	23833	24759	1133	1291
AVG	**35279.1**	**35094.5**	**35314.7**	**35.6**	**11609.8**	**11159.4**	**10746.2**	**-863.6**	**23669.3**	**23935.1**	**24568.9**	**899.6**	**1763.2**
SEM	**2994.0**	**2929.6**	**2816.8**	**249.5**	**1598.2**	**1614.7**	**1606.1**	**207.7**	**1780.8**	**1666.4**	**1666.4**	**247.5**	**382.9**
CHO													
CF	35231	34403	36298	1067	3016	3721	4006	990	32216	30682	32292	76	-914
GM	28345	27269	27364	-981	11695	11471	10876	-819	16650	15798	16488	-162	657
JQ	24114	23285	23809	-305	8996	8233	8109	-887	15119	15052	15701	582	1469
KH	28220	28325	28784	564	5742	5049	5571	-171	22477	23276	23213	736	907
LM	30944	29489	29290	-1654	11936	11461	10703	-1233	19008	18029	18587	-421	812
MM	38546	37756	37400	-1146	11356	10356	9549	-1807	27191	27400	27851	660	2467
NF	28641	28592	28811	170	6930	7282	7400	470	21711	21310	21411	-300	-770
RC	41232	38140	39130	-2102	14306	14530	13733	-573	26926	23610	25397	-1529	-956
RM	37773	36054	37837	64	12307	10156	11519	-788	25466	25898	26319	853	1641
TR	34515	34828	34625	110	15291	15411	14443	-848	19225	19417	20182	957	1805
AVG	**32756.1**	**31814.1**	**32334.8**	**-421.3**	**10157.5**	**9767.0**	**9590.9**	**-566.6**	**22598.9**	**22047.2**	**22744.1**	**145.2**	**711.8**
SEM	**1747.0**	**1600.2**	**1682.4**	**320.6**	**1235.6**	**1193.3**	**1062.8**	**256.7**	**1689.6**	**1611.0**	**1672.1**	**244.6**	**385.6**
PLA													
AR	25669	25678	26378	709	11081	10380	9826	-1255	14588	15298	16552	1964	3219
BM	32232	31709	30794	-1438	13155	12622	11672	-1483	19077	19087	19122	45	1528

DT	25461	24575	25776	315	8212	7545	8333	121	17249	17030	17443	194	73
JH	34301	33142	30346	-3955	4194	3748	3202	-992	30107	29394	30364	257	1249
JL	27838	28506	28052	214	9229	9448	9011	-218	18609	19058	19041	432	650
JM	47528	47433	46661	-867	18771	17735	17947	-824	28757	29699	28714	-43	781
JO	31406	30550	31123	-283	11786	11526	10969	-817	19620	19024	20154	534	1351
JW	30423	30777	30988	565	5532	5655	5457	-75	24891	25123	25531	640	715
MN	30093	28222	29518	-575	12021	11182	11922	-99	18072	17040	17595	-477	-378
VR	31316	30964	30814	-502	13068	12302	12087	-981	18247	18662	18727	480	1461
AVG	**31626.7**	**31155.6**	**31045.0**	**-581.7**	**10704.9**	**10214.3**	**10042.6**	**-662.3**	**20921.7**	**20941.5**	**21324.3**	**402.6**	**1064.9**
SEM	**1978.2**	**1996.4**	**1841.8**	**430.9**	**1321.8**	**1245.9**	**1271.7**	**175.1**	**1635.9**	**1646.1**	**1577.4**	**202.3**	**308.5**

Study 4			White Blood Cell #s (x10^3/(μL)						
Sub	Tmt Grp	Train Grp	WBC Base	WBC Mid	WBC End	WBC TT2 pre	WBC TT2 post	WBC TT4 pre	WBC TT4 post
PD	1	1	5.2	4.7	5.7	6.5	9.1	6.2	8.9
YL	1	1	4.2	3.4	3.9	5.2	9.8	4.5	8.7
PB	1	2	6.7	10.8	8.4	9.4	9	7.6	9.8
JF	1	2	4.7	4.7	4.9	5.7	7.1	4.5	6.4
NG	1	4	7.2	8.4	6.1	8.7	13.7	8.4	12.6
DIT	1	3	5.8	4.2	5.1	6.7	15	6.1	14.8
EV	1	4	5.6	5.2	2.2	6	11.5	6.7	12.1
MF	1	3	4.5	3.7	4.4	4.8	9.2	3.7	7.8
MP	1	3	7.5	5.7	5.4	6.2	11.2	5.6	7.8
VE	1	3	4	5	5.9	5.4	9.3	6.2	11.2
CG	1	4	6.1	7.5	7	6.3	9.9	8.2	12.3
LM	2	4	5.7	5.1	6.8	5.7	8.6	5.8	10.1
NF	2	4	8.1	7.5	5.6	7.4	10.5	8.3	12.7
RM	2	4	7.8	5.5	6.2	6	7.5	6.6	10.5
JQ	2	4	5.4	4.9	4.2	6	10.2	6.7	12.3
TR	2	4	3.5	5.1	6.7	5.3		6.5	7.4
SB	2	3	8.6	6	7.2	6.3		6.7	12.2
KH	2	2	4.7	6.5	5.3	5.6		6.9	6.1
MM	2	2	6.4	6.5	5.2	8.2	8.9	6.7	9
RC	2	1	5.1	5.2	5.4	5.3	9.6	7.2	9.2
CF	2	1	5.5	5.4	8.9	5.7	8.6	4.9	10
JO	3	1	2.9	4.8	4.5		8.3		6.8
MN	3	1	6.8	6.3	6.2	6	9.4	4.6	10.8
DT	3	2	5.6		2.5		7.7		
JM	3	2	6.9	3.6	6.2	7.1	9.9	6.8	10.5
AR	3	3	9	8.8	8.3	8.4	13.2	7.6	9.8
BM	3	3	4.8	3.5	3.7	3.4	6.7		7.6
JL	3	3	4.6	5.4	6.8	5.6	21.6	4.8	16.8
VR	3	3	8.3	5	5.7	6.4	7.7	5.2	11.2
JH	3	4	4.1	3.4	4.5	5.2	8.4	5.3	8.8
JW	3	4	8.4	4.4	7.3	8.5	9.1	7.3	9.9
		CM	**5.59**	**5.75**	**5.36**	**6.45**	**10.44**	**6.15**	**10.22**
		SEM	**0.36**	**0.68**	**0.49**	**0.43**	**0.69**	**0.46**	**0.77**
		CHO	**6.08**	**5.77**	**6.15**	**6.15**	**9.13**	**6.63**	**9.95**
		SEM	**0.52**	**0.26**	**0.42**	**0.30**	**0.33**	**0.28**	**0.68**
		PLA	**6.14**	**5.02**	**5.57**	**6.33**	**10.20**	**5.94**	**10.24**
		SEM	**0.65**	**0.54**	**0.56**	**0.54**	**1.38**	**0.40**	**0.91**

Study 4			Neutrophil #s (x10^3/(μL)						
Sub	Tmt Grp	Train Grp	NPs Base	NPs Mid	NPs End	NPs TT2 pre	NPs TT2 post	NPs TT4 pre	NPs TT4 post
PD	1	1	1.8	2	2.7	3.2	4.2	2.8	4.1
YL	1	1	1.3	1	0.9	3.2	5.4	2.3	3.9
PB	1	2	2.7	5	4	4.1	4.8	3.3	4.8
JF	1	2	2.2	2.4	2.3	3	3.3	2	2.2
NG	1	4	4.7	6.1	3.1	5.7	8.1	5.4	7.8
DIT	1	3	2.7	0.6	1.8	2.5	9.2	2.8	7.8
EV	1	4	2.7	3.1	1.2	3.4	6.7	3.8	8
MF	1	3	2.5	2	2.2	3.1	5.4	1.4	2.5
MP	1	3	3.8	2.1	1.7	3.3	7.3	2.3	4.8
VE	1	3	0.9	2.2	2.5	2.1	2.9	2.2	3.9
CG	1	4	2.6	4.4	3.2	2.9	4.8	4.4	5.8
LM	2	4	2.2	2	3.3	2.6	3.6	2.8	4.9
NF	2	4	2.1	3.7	2.1	3.7	5.4	3.2	4.8
RM	2	4	4.1	2.6	3.5	3	4.3	3.1	4.1
JQ	2	4	1.6	2.3	1.3	3.4	5.2	3.4	5.7
TR	2	4	1.2	2.3	3.3	2.8		3.1	3.3
SB	2	3	5.5	3.2	3.8	3.3		3.8	7.7
KH	2	2	1.1	2.7	1.3	2		1.9	1.5
MM	2	2	3	3.1	2.5	4.2	4.2	2.8	3.6
RC	2	1	1.3	2.3	1.6	2.3	3.6	3.7	4
CF	2	1	2.9	3	6.6	2.6	4	2.4	4.5
JO	3	1	1.1	2.7	2		4.6		2.7
MN	3	1	3.1	2.5	2.8	2.5	5	1.7	6.3
DT	3	2	2.7		0.9		4.3		
JM	3	2	3.7	1.6	3	3.8	5.2	3.9	4.9
AR	3	3	5.5	4.3	5.1	4.9	8.2	3.9	6.4
BM	3	3	2.9	1.5	1.7	1.3	2.3		3.3
JL	3	3	2.5	3	4.3	3.4	18.12	1.7	12.4
VR	3	3	3	2.2	2.5	2.7	4.2	2.7	7.5
JH	3	4	2	1.4	1.8	2.5	3.6	2.3	3.8
JW	3	4	3.9	1.3	2.8	3.9	5	2.4	3
		CM	**2.54**	**2.81**	**2.33**	**3.32**	**5.65**	**2.97**	**5.05**
		SEM	**0.32**	**0.51**	**0.28**	**0.28**	**0.60**	**0.35**	**0.62**
		CHO	**2.50**	**2.72**	**2.93**	**2.99**	**4.33**	**3.02**	**4.41**
		SEM	**0.45**	**0.17**	**0.50**	**0.21**	**0.23**	**0.18**	**0.51**
		PLA	**3.04**	**2.28**	**2.69**	**3.13**	**6.05**	**2.66**	**5.59**
		SEM	**0.37**	**0.31**	**0.39**	**0.35**	**1.42**	**0.29**	**0.97**

Study 4			Monocyte #s (x10^3/(μL)						
Sub	Tmt Grp	Train Grp	MO Base	MO Mid	MO End	MOs TT2 pre	MOs TT2 post	MOs TT4 pre	MOs TT4 post
PD	1	1	0.6	0.4	0.5	0.5	0.7	0.6	0.7
YL	1	1	0.4	0.3	0.4	0.3	0.5	0.3	0.5
PB	1	2	0.7	0.9	0.7	0.7	0.8	0.5	0.8
JF	1	2	0.4	0.4	0.7	0.4	0.6	0.5	0.6
NG	1	4	0.4	0.3	0.7	0.5	1	0.5	0.5
DIT	1	3	0.4	0.4	0.3	0.4	0.5	0.4	0.7
EV	1	4	0.5	0.2	0.2	0.4	0.7	0.5	0.2
MF	1	3	0.2	0.1	0.3	0.2	0.4	0.2	0.5
MP	1	3	0.6	0.6	0.7	0.4	0.6	0.5	0.5
VE	1	3	0.3	0.4	0.4	0.3	0.5	0.4	0.9
CG	1	4	0.6	0.3	0.4	0.6	0.9	0.7	0.7
LM	2	4	0.3	0.3	0.5	0.5	0.8	0.4	0.7
NF	2	4	0.8	0.5	0.8	0.7	0.8	0.8	1.3
RM	2	4	0.5	0.4	0.6	0.5	0.6	0.5	0.9
JQ	2	4	0.6	0.2	0.8	0.5	0.8	0.5	1.1
TR	2	4	0.2	0.3	0.3	0.4		0.3	0.4
SB	2	3	0.5	0.5	0.4	0.4		0.5	0.7
KH	2	2	0.6	0.5	0.6	0.5		0.6	0.5
MM	2	2	0.6	0.4	0.5	0.4	0.6	0.5	0.5
RC	2	1	0.4	0.5	0.6	0.3	0.6	0.5	0.8
CF	2	1	0.6	0.5	0.7	0.6	0.9	0.5	1.1
JO	3	1	0.2	0.2	0.2		0.2		0.3
MN	3	1	0.6	0.5	0.4	0.5	0.8	0.6	1.1
DT	3	2	0.6		0.4		0.6		
JM	3	2	0.3	0.4	0.3	0.5	0.6	0.3	0.5
AR	3	3	0.5	0.1	0.4	0.4	0.7	0.5	0.5
BM	3	3	0.3	0.3	0.3	0.3	0.7		0.8
JL	3	3	0.3	0.4	0.5	0.3	1.3	0.4	1
VR	3	3	0.7	0.5	0.4	0.7	0.8	0.4	0.8
JH	3	4	0.3	0.5	0.7	0.5	0.8	0.5	0.9
JW	3	4	0.7	0.6	0.8	0.7	0.7	0.7	1.1
		CM	**0.46**	**0.39**	**0.48**	**0.43**	**0.65**	**0.46**	**0.60**
		SEM	**0.05**	**0.06**	**0.06**	**0.04**	**0.06**	**0.04**	**0.06**
		CHO	**0.51**	**0.41**	**0.58**	**0.48**	**0.73**	**0.51**	**0.80**
		SEM	**0.05**	**0.03**	**0.05**	**0.04**	**0.04**	**0.04**	**0.09**
		PLA	**0.45**	**0.39**	**0.44**	**0.49**	**0.72**	**0.49**	**0.78**
		SEM	**0.06**	**0.05**	**0.06**	**0.05**	**0.09**	**0.04**	**0.09**

Citrate synthase activity (μmol/g/min)			
Subject	Base	Mid	End
CM			
PD	25.425	27.878	34.046
PB	14.607	19.960	21.580
YL	16.896	20.289	22.285
JF	11.963	17.339	18.718
DIT	20.4965	34.4895	42.269
VE	17.777	25.5055	27.331
MF	20.055	22.6605	30.5355
MP	16.66	19.682	29.275
CG	21.3555	27.827	21.94
EV	21.51	20.31	28.8585
NG	25	25.7955	31.2265
AVG	**19.25**	**23.79**	**28.01**
SEM	**1.25**	**1.52**	**2.04**
CHO			
RC	23.197	29.096	28.777
KH	11.493	16.423	13.071
MM	12.112	18.870	17.657
CF	23.673	24.839	27.420
GM	9.311	17.518	18.435
RM	15.6915	16.9185	23.7495
TR	18.2195	25.7875	21.7835
LM	24.666	30.978	37.831
NF	30.76	36.5595	34.606
JQ	21.7915	25.233	31.627
AVG	**19.09**	**24.22**	**25.50**
SEM	**2.08**	**2.04**	**2.40**
PLA			
DT	14.642	17.899	20.605
JM	16.987	17.142	20.458
JO	16.537	17.922	27.216
MN	17.314	20.468	24.730
AR	14.98	24.877	24.5345
BM	22.42	31.0105	31.186
JL	15.87	22.085	24.0565
VR	17.7045	21.8035	27.78
JH	21.3045	24.3515	29.3
JW	20.2025	24.917	25.388
AVG	**17.80**	**22.25**	**25.53**
SEM	**0.84**	**1.34**	**1.10**

Succinate dehydrogenase activity (μmol/g/min)			
Subject	Base	Mid	End
CM			
PD	4.425	4.954	5.883
PB	3.186	2.819	3.763
YL	4.013	4.337	5.569
JF	3.733	5.416	6.349
DIT	4.81	6.216	7.477
VE	4.262	6.262	5.269
MF	5.309	6.135	6.748
MP	5.032	3.755	6.108
CG	4.283	4.616	4.982
EV	4.449	5.959	5.454
NG	6.057	6.827	7.709
AVG	**4.51**	**5.21**	**5.94**
SEM	**0.24**	**0.37**	**0.34**
CHO			
RC	4.219	4.471	4.572
KH	3.597	3.185	3.359
MM	3.534	3.281	4.158
CF	4.399	5.745	5.266
GM	2.906	3.663	3.984
RM	3.665	4.674	7.104
TR	4.969	5.014	5.569
LM	4.775	6.609	6.607
NF	4.444	9.896	8.056
JQ	5.684	6.665	7.139
AVG	**4.22**	**5.32**	**5.58**
SEM	**0.25**	**0.61**	**0.48**
PLA			
DT	2.585	3.719	4.596
JM	4.465	4.502	5.792
JO	3.515	3.784	5.09
MN	5.024	4.594	5.079
AR	3.174	4.805	4.941
BM	5.296	4.822	6.362
JL	4.313	4.156	5.776
VR	4.705	3.985	5.015
JH	5.798	4.604	6.874
JW	5.846	5.67	6.918
AVG	**4.47**	**4.46**	**5.64**
SEM	**0.35**	**0.18**	**0.26**

Study 4		
PGC-1α, % of standard		
CM		
Subject	**Base**	**End**
CG	84.7	139.2
DIT	117.3	342.5
EV	90.0	181.2
JF	65.6	120.2
MF	86.3	260.6
MP	156.1	240.3
NG	172.4	215.1
PB	62.5	90.2
PD	97.9	105.6
VE	169.3	178.0
YL	101.4	108.2
AVE	**109.40**	**180.10**
SE	**11.92**	**23.70**
CHO		
Subject	**Base**	**End**
CF	141.4	253.3
GM	88.0	125.7
JQ	111.6	183.2
KH	68.7	91.7
LM	217.4	229.8
MM	80.0	105.2
NF	120.5	203.2
RC	115.6	165.7
RM	122.5	142.5
TR	172.7	260.5
AVE	**123.84**	**176.08**
SE	**14.11**	**19.05**
PLA		
Subject	**Base**	**End**
AR	71.6	138.9
BM	178.5	219.5
DT	105.3	183.8
JH	134.9	184.8
JL	103.4	201.8
JM	63.5	87.1
JO	95.1	107.2
JW	117.2	161.7
MN	36.9	249.8
VR	109.7	142.9
AVE	**101.61**	**167.75**
SE	**12.44**	**15.90**

Works Cited

Ahlborg B, Bergstrom J, Ekelund LG, and Hultman E. Muscle glycogen and muscle electrolytes during prolonged physical exercise. Acta Physiol Scand 70: 129-142, 1967.

Akerstrom T, Steensberg A, Keller P, Keller C, Penkowa M, and Pedersen BK. Exercise induces interleukin-8 expression in human skeletal muscle. J Physiol 563: 507-516, 2005.

Akira S, Taga T, and Kishimoto T. Interleukin-6 in biology and medicine. Adv Immunol 54: 1-78, 1993.

Anthony TG, McDaniel BJ, Knoll P, Bunpo P, Paul GL, and McNurlan MA. Feeding Meals Containing Soy or Whey Protein after Exercise Stimulates Protein Synthesis and Translation Initiation in the Skeletal Muscle of Male Rats. J Nutr 137: 357-362, 2007.

Armstrong JL, Bonavaud SM, Toole BJ, and Yeaman SJ. Regulation of Glycogen Synthesis by Amino Acids in Cultured Human Muscle Cells. J Biol Chem 276: 952-956, 2001.

Arnold L, Henry A, Poron F, Baba-Amer Y, van Rooijen N, Plonquet A, Gherardi RK, and Chazaud B. Inflammatory monocytes recruited after skeletal muscle injury switch into antiinflammatory macrophages to support myogenesis. J Exp Med 204: 1057-1069, 2007.

Åstrand PO, and Rodahl K. Textbook of work physiology: Physiological bases of exercise. . New York: McGraw-Hill Book Company, 1977.

Atwater W. Coefficients of digestibility and availability of the nutrients of food. Proceedings of the American Physiology Society 30: 14-19, 1909.

Avni D, Biberman Y, and Meyuhas O. The 5' terminal oligopyrimidine tract confers translational control on TOP mRNAs in a cell type- and sequence context-dependent manner. Nucleic Acids Res 25: 995-1001, 1997.

Baar K, Wende AR, Jones TE, Marison M, Nolte LA, Chen MAY, Kelly DP, and Holloszy JO. Adaptations of skeletal muscle to exercise: rapid increase in the transcriptional coactivator PGC-1. FASEB J 16: 1879-1886, 2002.

Baldwin KM, Klinkerfuss GH, Terjung RL, Mole PA, and Holloszy JO. Respiratory capacity of white, red, and intermediate muscle: adaptative response to exercise. Am J Physiol -- Legacy Content 222: 373-378, 1972.

Bartholomew K, O'Brien BJ, and Gill ND. Plasma volume expansion 24-hours post-exercise: effect of doubling the volume of replacement fluid. Journal of Sports Science and Medicine 4: 179-184, 2005.

Baslund B, Lyngberg K, Andersen V, Halkjaer Kristensen J, Hansen M, Klokker M, and Pedersen BK. Effect of 8 wk of bicycle training on the immune system of patients with rheumatoid arthritis. J Appl Physiol 75: 1691-1695, 1993.

Baty JJ, Hwang H, Ding Z, Bernard JR, Wang B, Kwon B, and Ivy JL. The effect of a carbohydrate and protein supplement on resistance exercise performance, hormonal response, and muscle damage. J Strength Cond Res 21: 321–329, 2007.
Benzi G, Panceri P, de Bernardi M, Villa R, Arcelli E, D'Angelo L, Arrigoni E, and Berte F. Mitochondrial enzymatic adaptation of skeletal muscle to endurance training. J Appl Physiol 38: 565-569, 1975.
Berardi JM, Noreen EE, and Lemon PW. Recovery from a cycling time trial is enhanced with carbohydrate-protein supplementation vs. isoenergetic carbohydrate supplementation. J Int Soc Sports Nutr 5: 24, 2008.
Berardi JM, Price TB, Noreen EE, and Lemon PW. Postexercise muscle glycogen recovery enhanced with a carbohydrate-protein supplement. Med Sci Sports Exerc 38: 1106-1113, 2006.
Bergstrom J, Hermansen L, Hultman E, and Saltin B. Diet, muscle glycogen and physical performance. Acta Physiol Scand 71: 140-150, 1967.
Bergström J, and Hultman E. Muscle glycogen synthesis after exercise: an enhancing factor localized to the muscle cells in man. Nature 210: 309-310, 1966.
Biolo G, Tipton KD, Klein S, and Wolfe RR. An abundant supply of amino acids enhances the metabolic effect of exercise on muscle protein. Am J Physiol Endocrinol Metab 273: E122-129, 1997.
Biolo G, Williams BD, Fleming RY, and Wolfe RR. Insulin action on muscle protein kinetics and amino acid transport during recovery after resistance exercise. Diabetes 48: 949-957, 1999.
Blannin AK, Chatwin LJ, Cave R, and Gleeson M. Effects of submaximal cycling and long-term endurance training on neutrophil phagocytic activity in middle aged men. Br J Sports Med 30: 125-129, 1996.
Blom P, Høstmark A, Vaage O, Kardel K, and Maehlum S. Effect of different post-exercise sugar diets on the rate of muscle glycogen synthesis. Med Sci Sports Exc 19: 491-496, 1987.
Blomstrand E, Eliasson J, Karlsson HKR, and Kohnke R. Branched-Chain Amino Acids Activate Key Enzymes in Protein Synthesis after Physical Exercise. J Nutr 136: 269S-273, 2006.
Bolster DR, Jefferson LS, and Kimball SR. Regulation of protein synthesis associated with skeletal muscle hypertrophy by insulin-, amino acid- and exercise-induced signalling. Proc Nutr Soc 63: 351-356, 2004.
Borsheim E, Cree MG, Tipton KD, Elliott TA, Aarsland A, and Wolfe RR. Effect of carbohydrate intake on net muscle protein synthesis during recovery from resistance exercise. J Appl Physiol 96: 674-678, 2004.
Borsheim E, Tipton KD, Wolf SE, and Wolfe RR. Essential amino acids and muscle protein recovery from resistance exercise. Am J Physiol Endocrinol Metab 283: E648-657, 2002.
Bowles DK, Woodman CR, and Laughlin MH. Coronary smooth muscle and endothelial adaptations to exercise training. Exerc Sport Sci Rev 28: 57-62, 2000.

Brancaccio P, Maffulli N, and Limongelli FM. Creatine kinase monitoring in sport medicine. Br Med Bull 81-82: 209-230, 2007.
Breen L, Tipton KD, and Jeukendrup AE. No effect of carbohydrate-protein on cycling performance and indices of recovery. Med Sci Sports Exerc 42: 1140-1148, 2010.
Bruunsgaard H, Galbo H, Halkjaer-Kristensen J, Johansen TL, MacLean DA, and Pedersen BK. Exercise-induced increase in serum interleukin-6 in humans is related to muscle damage. J Physiol 499: 833-841, 1997.
Calders P, Matthys D, Derave W, and Pannier JL. Effect of branched-chain amino acids (BCAA), glucose, and glucose plus BCAA on endurance performance in rats. Med Sci Sports Exerc 31: 583-587, 1999.
Campbell PT, Wener MH, Sorensen B, Wood B, Chen-Levy Z, Potter JD, McTiernan A, and Ulrich CM. Effect of exercise on in vitro immune function: a 12-month randomized, controlled trial among postmenopausal women. J Appl Physiol 104: 1648-1655, 2008.
Carrithers JA, Williamson DL, Gallagher PM, Godard MP, Schulze KE, and Trappe SW. Effects of postexercise carbohydrate-protein feedings on muscle glycogen restoration. J Appl Physiol 88: 1976-1982, 2000.
Carter SL, Rennie CD, Hamilton SJ, and Tarnopolsky MA. Changes in skeletal muscle in males and females following endurance training. Can J Physiol Pharmacol 79: 386-392, 2001.
Casey A, Short AH, Hultman E, and Greenhaff PL. Glycogen resynthesis in human muscle fibre types following exercise-induced glycogen depletion. J Physiol 483: 265-271, 1995.
Cassatella MA, Meda L, Gasperini S, Calzetti F, and Bonora S. Interleukin 10 (IL-10) upregulates IL-1 receptor antagonist production from lipopolysaccharide-stimulated human polymorphonuclear leukocytes by delaying mRNA degradation. J Exp Med 179: 1695-1699, 1994.
Castell LM, Poortmans JR, and Newsholme EA. Does glutamine have a role in reducing infections in athletes? Eur J Appl Phys Occup Physiol 73: 488-490, 1996.
Cermak NM, Solheim AS, Gardner MS, Tarnopolsky MA, and Gibala MJ. Muscle metabolism during exercise with carbohydrate or protein-carbohydrate ingestion. Med Sci Sports Exerc 41: 2158-2164, 2009.
Chernoff AE, Granowitz EV, Shapiro L, Vannier E, Lonnemann G, Angel JB, Kennedy JS, Rabson AR, Wolff SM, and Dinarello CA. A randomized, controlled trial of IL-10 in humans. Inhibition of inflammatory cytokine production and immune responses. J Immunol 154: 5492-5499, 1995.
Chubak J, McTiernan A, Sorensen B, Wener MH, Yasui Y, Velasquez M, Wood B, Rajan KB, Wetmore CM, Potter JD, and Ulrich CM. Moderate-intensity exercise reduces the incidence of colds among postmenopausal women. Am J Med 119: 937-942, 2006.
Ciechanover A. The ubiquitin-proteasome proteolytic pathway. Cell 79: 13-21, 1994.

Ciechanover A, and Schwartz AL. The ubiquitin-mediated proteolytic pathway: mechanisms of recognition of the proteolytic substrate and involvement in the degradation of native cellular proteins. FASEB J 8: 182-191, 1994.
Clarkson PM, and Hubal MJ. Exercise-induced muscle damage in humans. Am J Phys Med Rehabil 81: S52-S69, 2002.
Cockburn E, Hayes PR, French DN, Stevenson E, and Gibson ASC. Acute milk-based protein-CHO supplementation attenuates exercise-induced muscle damage. Applied Physiology, Nutrition & Metabolism 33: 775-783, 2008.
Cockburn E, Hayes PR, French DN, Stevenson E, and St Clair Gibson A. Acute milk-based protein-CHO supplementation attenuates exercise-induced muscle damage. Appl Physiol Nutr Metab 33: 775-783, 2008.
Coggan AR, and Coyle EF. Effect of carbohydrate feedings during high-intensity exercise. J Appl Physiol 65: 1703-1709, 1988.
Coggan AR, and Coyle EF. Reversal of fatigue during prolonged exercise by carbohydrate infusion or ingestion. J Appl Physiol 63: 2388-2395, 1987.
Combest T, Saunders M, Kane M, and Todd K. Attenuated CPK Following Carbohydrate/protein Intervention Improves Subsequent Performance. Med Sci Sports Exc 37: S42, 2005.
Convertino VA. Blood volume: its adaptation to endurance training. Med Sci Sports Exerc 23: 1338-1348, 1991.
Convertino VA, Brock PJ, Keil LC, Bernauer EM, and Greenleaf JE. Exercise training-induced hypervolemia: role of plasma albumin, renin, and vasopressin. J Appl Physiol 48: 665-669, 1980.
Costill DL, Pascoe DD, Fink WJ, Robergs RA, Barr SI, and Pearson D. Impaired muscle glycogen resynthesis after eccentric exercise. J Appl Phys 69: 46-50, 1990.
Coyle EF, Coggan AR, Hopper MK, and Walters TJ. Determinants of endurance in well-trained cyclists. J Appl Physiol 64: 2622-2630, 1988.
Coyle EF, Hemmert MK, and Coggan AR. Effects of detraining on cardiovascular responses to exercise: role of blood volume. J Appl Physiol 60: 95-99, 1986.
Coyle EF, Martin WH, 3rd, Sinacore DR, Joyner MJ, Hagberg JM, and Holloszy JO. Time course of loss of adaptations after stopping prolonged intense endurance training. J Appl Physiol 57: 1857-1864, 1984.
Cribb PJ, and Hayes A. Effects of supplement timing and resistance exercise on skeletal muscle hypertrophy. Med Sci Sports Exerc 38: 1918-1925, 2006.
Cross DA, Alessi DR, Cohen P, Andjelkovich M, and Hemmings BA. Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase B. Nature 378: 785-789, 1995.
Currell K, and Jeukendrup AE. Superior endurance performance with ingestion of multiple transportable carbohydrates. Med Sci Sports Exerc 40: 275-281, 2008.
Davis JA, Vodak P, Wilmore JH, Vodak J, and Kurtz P. Anaerobic threshold and maximal aerobic power for three modes of exercise. J Appl Phys 41:544-550, 1976.
De Bock K, Derave W, Eijnde BO, Hesselink MKC, Koninckx E, Rose AJ, Schrauwen P, Bonen A, Richter EA, and Hespel PJ. Effect of training in the fasted

state on metabolic responses during exercise with carbohydrate intake. J Appl Physiol 01195.02007, 2008.
Doi T, Matsuo T, Sugawara M, Matsumoto K, Minehira K, Hamada K, Okamura K, and Suzuki M. New approach for weight reduction by a combination of diet, light resistance exercise and the timing of ingesting a protein supplement. Asia Pac J Clin Nutr 10: 226-232, 2001.
Doyle JA, Sherman WM, and Strauss RL. Effects of eccentric and concentric exercise on muscle glycogen replenishment. J Appl Physiol 74: 1848-1855, 1993.
Dreyer HC, Drummond MJ, Pennings B, Fujita S, Glynn EL, Chinkes DL, Dhanani S, Volpi E, and Rasmussen BB. Leucine-enriched essential amino acid and carbohydrate ingestion following resistance exercise enhances mTOR signaling and protein synthesis in human muscle. Am J Physiol Endo Metab 294: E392-400, 2008.
Driessen-Kletter MF, Amelink GJ, Bar PR, and van Gijn J. Myoglobin is a sensitive marker of increased muscle membrane vulnerability. J Neurol 237: 234-238, 1990.
Drummond MJ, Glynn EL, Fry CS, Dhanani S, Volpi E, and Rasmussen BB. Essential Amino Acids Increase MicroRNA-499, -208b, and -23a and Downregulate Myostatin and Myocyte Enhancer Factor 2C mRNA Expression in Human Skeletal Muscle. J Nutr 139: 2279-2284, 2009.
Dufaux B, and Order U. Complement activation after prolonged exercise. Clin Chim Acta 179: 45-49, 1989.
Duncombe WG. The colorimetric micro-determination of non-esterified fatty acids in plasma. Clin Chim Acta 9: 122-125, 1964.
Dupont-Versteegden EE, Fluckey JD, Knox M, Gaddy D, and Peterson CA. Effect of flywheel-based resistance exercise on processes contributing to muscle atrophy during unloading in adult rats. J Appl Physiol 101: 202-212, 2006.
Ekblom B, Astrand PO, Saltin B, Stenberg J, and Wallstrom B. Effect of training on circulatory response to exercise. J Appl Physiol 24: 518-528, 1968.
Elliot TA, Cree MG, Sanford AP, Wolfe RR, and Tipton KD. Milk ingestion stimulates net muscle protein synthesis following resistance exercise. Med Sci Sports Exerc 38: 667-674, 2006.
Escobar J, Frank JW, Suryawan A, Nguyen HV, Kimball SR, Jefferson LS, and Davis TA. Physiological rise in plasma leucine stimulates muscle protein synthesis in neonatal pigs by enhancing translation initiation factor activation. Am J Physiol Endocrinol Metab 288: E914-921, 2005.
Esmarck B, Andersen JL, Olsen S, Richter EA, Mizuno M, and Kjar M. Timing of postexercise protein intake is important for muscle hypertrophy with resistance training in elderly humans. J Physiol 535: 301-311, 2001.
Espersen GT, Elbaek A, Ernst E, Toft E, Kaalund S, Jersild C, and Grunnet N. Effect of physical exercise on cytokines and lymphocyte subpopulations in human peripheral blood. APMIS 98: 395-400, 1990.
Evans WJ. Effects of Exercise on Senescent Muscle. Clin-Orthop 403 Suppl: S211-220, 2002.

Evans WJ, Meredith CN, Cannon JG, Dinarello CA, Frontera WR, Hughes VA, Jones BH, and Knuttgen HG. Metabolic changes following eccentric exercise in trained and untrained men. J Appl Physiol 61: 1864-1868, 1986.
Farrell PA, Wilmore JH, Coyle EF, Billing JE, and Costill DL. Plasma lactate accumulation and distance running performance. Med Sci Sports 11: 338-344, 1979.
Febbraio MA, Hiscock N, Sacchetti M, Fischer CP, and Pedersen BK. Interleukin-6 Is a Novel Factor Mediating Glucose Homeostasis During Skeletal Muscle Contraction. Diabetes 53: 1643-1648, 2004.
Febbraio MA, and Pedersen BK. Muscle-derived interleukin-6: mechanisms for activation and possible biological roles. FASEB J 16: 1335-1347, 2002.
Fedele MJ, Hernandez JM, Lang CH, Vary TC, Kimball SR, Jefferson LS, and Farrell PA. Severe diabetes prohibits elevations in muscle protein synthesis after acute resistance exercise in rats. J Appl Physiol 88: 102-108, 2000.
Fernstrom M, Tonkonogi M, and Sahlin K. Effects of acute and chronic endurance exercise on mitochondrial uncoupling in human skeletal muscle. J Physiol 554: 755-763, 2004.
Flakoll PJ, Judy T, Flinn K, Carr C, and Flinn S. Postexercise protein supplementation improves health and muscle soreness during basic military training in marine recruits. J Appl Physiol 96: 951-956, 2004.
Fluckey JD, Vary TC, Jefferson LS, and Farrell PA. Augmented insulin action on rates of protein synthesis after resistance exercise in rats. Am J Physiol Endocrinol Metab 270: E313-319, 1996.
Ford ES. Does exercise reduce inflammation? Physical activity and C-reactive protein among U.S. adults. Epidemiology 13: 561-568, 2002.
Frayn KN. Calculation of substrate oxidation rates in vivo from gaseous exchange. J Appl Physiol 55: 628-634, 1983.
Frydelund-Larsen L, Penkowa M, Akerstrom T, Zankari A, Nielsen S, and Pedersen BK. Muscle: Exercise induces interleukin-8 receptor (CXCR2) expression in human skeletal muscle. Exp Physiol 92: 233-240, 2007.
Gabriel H, and Kindermann W. The acute immune response to exercise: what does it mean? Int J Sports Med 18: S28-45, 1997.
Gabriel H, Urhausen A, and Kindermann W. Circulating leucocyte and lymphocyte subpopulations before and after intensive endurance exercise to exhaustion. Eur J Appl Physiol Occup Physiol 63: 449-457, 1991.
Gautsch TA, Anthony JC, Kimball SR, Paul GL, Layman DK, and Jefferson LS. Availability of eIF4E regulates skeletal muscle protein synthesis during recovery from exercise. Am J Physiol Cell Physiol 274: C406-414, 1998.
Gillen CM, Lee R, Mack GW, Tomaselli CM, Nishiyasu T, and Nadel ER. Plasma volume expansion in humans after a single intense exercise protocol. J Appl Physiol 71: 1914-1920, 1991.
Gingras A-C, Gygi SP, Raught B, Polakiewicz RD, Abraham RT, Hoekstra MF, Aebersold R, and Sonenberg N. Regulation of 4E-BP1 phosphorylation: a novel two-step mechanism. Genes & Dev 13: 1422-1437, 1999.

Gleeson M. Immune function in sport and exercise. J Appl Physiol 103: 693-699, 2007.
Gleeson M. The scientific basis of practical strategies to maintain immunocompetence in elite athletes. Exercise Immunology Review 6: 75-101, 2000.
Glover EI, Oates BR, Tang JE, Moore DR, Tarnopolsky MA, and Phillips SM. Resistance exercise decreases eIF2B{varepsilon} phosphorylation and potentiates the feeding-induced stimulation of p70S6K1 and rpS6 in young men. Am J Physiol Regulatory Integrative Comp Physiol 295: R604-610, 2008.
Goetz FC, and Greenberg BZ. A simple immunoassay for small amounts of insulin. J Clin Lab Med 58: 819-822, 1961.
Goto M, Terada S, Kato M, Katoh M, Yokozeki T, Tabata I, and Shimokawa T. cDNA Cloning and mRNA analysis of PGC-1 in epitrochlearis muscle in swimming-exercised rats. Biochem Biophys Res Commun 274: 350-354, 2000.
Green HJ, Bombardier EB, Duhamel TA, Stewart RD, Tupling AR, and Ouyang J. Metabolic, Enzymatic and Transporter Responses in Human Muscle During Three Consecutive Days of Exercise and Recovery. Am J Physiol Regul Integr Comp Physiol 00171.02008, 2008.
Halson SL, Lancaster GI, Jeukendrup AE, and Gleeson M. Immunological responses to overreaching in cyclists. Med Sci Sports Exerc 35: 854-861, 2003.
Hansen JB, Wilsgard L, and Osterud B. Biphasic changes in leukocytes induced by strenuous exercise. Eur J Appl Physiol Occup Physiol 62: 157-161, 1991.
Hara K, Yonezawa K, Kozlowski MT, Sugimoto T, Andrabi K, Weng Q-P, Kasuga M, Nishimoto I, and Avruch J. Regulation of eIF-4E BP1 Phosphorylation by mTOR. J Biol Chem 272: 26457-26463, 1997.
Hartman JW, Tang JE, Wilkinson SB, Tarnopolsky MA, Lawrence RL, Fullerton AV, and Phillips SM. Consumption of fat-free fluid milk after resistance exercise promotes greater lean mass accretion than does consumption of soy or carbohydrate in young, novice, male weightlifters. Am J Clinical Nutrition 86: 373-381, 2007.
Henriksson J, and Reitman JS. Time course of changes in human skeletal muscle succinate dehydrogenase and cytochrome oxidase activities and maximal oxygen uptake with physical activity and inactivity. Acta Physiol Scand 99: 91-97, 1977.
Hermansen L, Hultman E, and Saltin B. Muscle glycogen during prolonged severe exercise. Acta Physiol Scand 71: 129-139, 1967.
Hickson RC, Bomze HA, and Holloszy JO. Linear increase in aerobic power induced by a strenuous program of endurance exercise. J Appl Physiol 42: 372-376, 1977.
Hiscock N, and Pedersen BK. Exercise-induced immunodepression- plasma glutamine is not the link. J Appl Physiol 93: 813-822, 2002.
Hochstrasser M. Ubiquitin, proteasomes, and the regulation of intracellular protein degradation. Curr Opin Cell Biol 7: 215-223, 1995.
Hoffman-Goetz L, and Pedersen BK. Exercise and the immune system: a model of the stress response? Immunol Today 15: 382-387, 1994.

Hohorst HJ. Determination of L-lactate with LDH and DPN. In: Methods of Enzymatic Analysis, edited by Bergmeyer HU. New York: Academic, 1963, p. 266-270.
Holloszy JO. Adaptation of skeletal muscle to endurance exercise. Med Sci Sports 7: 155-164, 1975.
Holloszy JO. Biochemical Adaptations in Muscle. Effects of exercise on mitochondrial oxygen uptake and respiratory enzyme activity in skeletal muscle. J Biol Chem 242: 2278-2282, 1967.
Holloszy JO, and Booth FW. Biochemical adaptations to endurance exercise in muscle. Annu Rev Physiol 38: 273-291, 1976.
Holloszy JO, and Coyle EF. Adaptations of skeletal muscle to endurance exercise and their metabolic consequences. J Appl Physiol 56: 831-838, 1984.
Holloszy JO, Oscai LB, Don IJ, and Mole PA. Mitochondrial citric acid cycle and related enzymes: adaptive response to exercise. Biochem Biophys Res Commun 40: 1368-1373, 1970.
Hopper MK, Coggan AR, and Coyle EF. Exercise stroke volume relative to plasma-volume expansion. J Appl Physiol 64: 404-408, 1988.
Howarth KR, Moreau NA, Phillips SM, and Gibala MJ. Coingestion of protein with carbohydrate during recovery from endurance exercise stimulates skeletal muscle protein synthesis in humans. J Appl Physiol 106: 1394-1402, 2009.
Huang H, and Tindall DJ. Dynamic FoxO transcription factors. J Cell Sci 120: 2479-2487, 2007.
Irrcher I, Adhihetty PJ, Sheehan T, Joseph A-M, and Hood DA. PPARgamma coactivator-1alpha expression during thyroid hormone- and contractile activity-induced mitochondrial adaptations. Am J Physiol Cell Physiol 284: C1669-1677, 2003.
Ivy JL. Regulation of muscle glycogen repletion, muscle protein synthesis and repair following exercise. J Sports Sci and Med 3: 131-138, 2004.
Ivy JL. Role of exercise training in the prevention and treatment of insulin resistance and non-insulin-dependent diabetes mellitus. Sports Med 24: 321-336, 1997.
Ivy JL, Ding Z, Hwang H, Cialdella-Kam LC, and Morrison PJ. Post exercise carbohydrate-protein supplementation: phosphorylation of muscle proteins involved in glycogen synthesis and protein translation. Amino Acids 35: 89-97, 2008.
Ivy JL, Goforth HW, Jr., Damon BM, McCauley TR, Parsons EC, and Price TB. Early postexercise muscle glycogen recovery is enhanced with a carbohydrate-protein supplement. J Appl Physiol 93: 1337-1344, 2002.
Ivy JL, and Holloszy JO. Persistent increase in glucose uptake by rat skeletal muscle following exercise. Am J Physiol Cell Physiol 241: C200-203, 1981.
Ivy JL, Katz AL, Cutler CL, Sherman WM, and Coyle EF. Muscle glycogen synthesis after exercise: effect of time of carbohydrate ingestion. J Appl Physiol 64: 1480-1485, 1988.

Ivy JL, and Kuo CH. Regulation of GLUT4 protein and glycogen synthase during muscle glycogen synthesis after exercise. Acta Physiologica Scandinavica 162: 295-304, 1998.
Ivy JL, Lee MC, Brozinick JT, Jr., and Reed MJ. Muscle glycogen storage after different amounts of carbohydrate ingestion. J Appl Physiol 65: 2018-2023, 1988.
Ivy JL, Miller W, Dover V, Goodyear LG, Sherman WM, Farrell S, and Williams H. Endurance improved by ingestion of a glucose polymer supplement. Med Sci Sports Exerc 15: 466-471, 1983.
Ivy JL, Res PT, Sprague RC, and Widzer MO. Effect of a carbohydrate-protein supplement on endurance performance during exercise of varying intensity. Int J Sport Nutr Exerc Metab 13: 382-395, 2003.
Ivy JL, Withers RT, Van Handel PJ, Elger DH, and Costill DL. Muscle respiratory capacity and fiber type as determinants of the lactate threshold. J Appl Physiol 48: 523-527, 1980.
Jenkins JK, Malyak M, and Arend WP. The effects of interleukin-10 on interleukin-1 receptor antagonist and interleukin-1 beta production in human monocytes and neutrophils. Lymphokine Cytokine Res 13: 47-54, 1994.
Jentjens RL, Achten J, and Jeukendrup AE. High oxidation rates from combined carbohydrates ingested during exercise. Med Sci Sports Exerc 36: 1551-1558, 2004.
Jentjens RL, and Jeukendrup AE. High rates of exogenous carbohydrate oxidation from a mixture of glucose and fructose ingested during prolonged cycling exercise. Br J Nutr 93: 485-492, 2005.
Jentjens RLPG, Moseley L, Waring RH, Harding LK, and Jeukendrup AE. Oxidation of combined ingestion of glucose and fructose during exercise. J Appl Physiol 96: 1277-1284, 2004.
Jentjens RLPG, van Loon LJC, Mann CH, Wagenmakers AJM, and Jeukendrup AE. Addition of protein and amino acids to carbohydrates does not enhance postexercise muscle glycogen synthesis. J Appl Physiol 91: 839-846, 2001.
Jentjens RLPG, Venables MC, and Jeukendrup AE. Oxidation of exogenous glucose, sucrose, and maltose during prolonged cycling exercise. J Appl Physiol 96: 1285-1291, 2004.
Jeukendrup AE. Carbohydrate intake during exercise and performance. Nutrition 20: 669-677, 2004.
Jeukendrup AE, and Jentjens R. Oxidation of carbohydrate feedings during prolonged exercise: current thoughts, guidelines and directions for future research. Sports Med 29: 407-424, 2000.
Jeukendrup AE, Wagenmakers AJM, Stegen JHCH, Gijsen AP, Brouns F, and Saris WHM. Carbohydrate ingestion can completely suppress endogenous glucose production during exercise. Am J Physiol Endocrinol Metab 276: E672-683, 1999.
Josse AR, Tang JE, Tarnopolsky MA, and Phillips SM. Body composition and strength changes in women with milk and resistance exercise. Med Sci Sports Exerc 42: 1122-1130, 2010.

Kahn SE, Larson VG, Beard JC, Cain KC, Fellingham GW, Schwartz RS, Veith RC, Stratton JR, Cerqueira MD, and Abrass IB. Effect of exercise on insulin action, glucose tolerance, and insulin secretion in aging. Am J Physiol Endocrinol Metab 258: E937-943, 1990.
Kammer L, Ding Z, Wang B, Hara D, Liao Y-H, and Ivy J. Cereal and nonfat milk support muscle recovery following exercise. Journal of the International Society of Sports Nutrition 6: 11, 2009.
Karlsson HKR, Nilsson P-A, Nilsson J, Chibalin AV, Zierath JR, and Blomstrand E. Branched-chain amino acids increase p70S6k phosphorylation in human skeletal muscle after resistance exercise. Am J Physiol Endocrinol Metab 287: E1-7, 2004.
Karp JR, Johnston JD, Tecklenburg S, Mickleborough TD, Fly AD, and Stager JM. Chocolate Milk as a Post-Exercise Recovery Aid. International Journal of Sport Nutrition and Exercise Metabolism 16: 78-91, 2006.
Kelly DP, and Scarpulla RC. Transcriptional regulatory circuits controlling mitochondrial biogenesis and function. Genes & Dev 18: 357-368, 2004.
Kim D-H, Sarbassov DD, Ali SM, King JE, Latek RR, Erdjument-Bromage H, Tempst P, and Sabatini DM. mTOR Interacts with Raptor to Form a Nutrient-Sensitive Complex that Signals to the Cell Growth Machinery. Cell 110: 163-175, 2002.
Kimball SR, Farrell PA, and Jefferson LS. Exercise Effects on Muscle Insulin Signaling and Action: Invited Review: Role of insulin in translational control of protein synthesis in skeletal muscle by amino acids or exercise. J Appl Physiol 93: 1168-1180, 2002.
Kimball SR, and Jefferson LS. Regulation of global and specific mRNA translation by oral administration of branched-chain amino acids. Biochem Biophys Res Commun 313: 423-427, 2004.
Kimball SR, and Jefferson LS. Signaling Pathways and Molecular Mechanisms through which Branched-Chain Amino Acids Mediate Translational Control of Protein Synthesis. J Nutr 136: 227S-231, 2006.
King DE, Carek P, Mainous AG, and Pearson WS. Inflammatory markers and exercise: differences related to exercise type. Med Sci Sports Exerc 35: 575-581, 2003.
Konig D, Schumacher YO, Heinrich L, Schmid A, Berg A, and Dickhuth HH. Myocardial stress after competitive exercise in professional road cyclists. Med Sci Sports Exerc 35: 1679-1683, 2003.
Koopman R, Wagenmakers AJM, Manders RJF, Zorenc AHG, Senden JMG, Gorselink M, Keizer HA, and van Loon LJC. Combined ingestion of protein and free leucine with carbohydrate increases postexercise muscle protein synthesis in vivo in male subjects. Am J Physiol Endocrinol Metab 288: E645-653, 2005.
Kramer HF, and Goodyear LJ. Exercise, MAPK, and NF-{kappa}B signaling in skeletal muscle. J Appl Physiol 103: 388-395, 2007.
Kreider RB, Fry AC, and O'Toole ML editors. Overtraining in Sport. Champaign, IL: Human Kinetics, 1998.

Krotkiewski M, Lonnroth P, Mandroukas K, Wroblewski Z, Rebuffe-Scrive M, Holm G, Smith U, and Bjorntorp P. The effects of physical training on insulin secretion and effectiveness and on glucose metabolism in obesity and type 2 (non-insulin-dependent) diabetes mellitus. Diabetologia 28: 881-890, 1985.
Krzywkowski K, Petersen EW, Ostrowski K, Link-Amster H, Boza J, Halkjaer-Kristensen J, and Pedersen BK. Effect of glutamine and protein supplementation on exercise-induced decreases in salivary IgA. J Appl Physiol 91: 832-838, 2001.
Lancaster GI, Khan Q, Drysdale PT, Wallace F, Jeukendrup AE, Drayson MT, and Gleeson M. Effect of prolonged exercise and carbohydrate ingestion on type 1 and type 2 T lymphocyte distribution and intracellular cytokine production in humans. J Appl Physiol 98: 565-571, 2005.
Laughlin MH, and McAllister RM. Exercise training-induced coronary vascular adaptation. J Appl Physiol 73: 2209-2225, 1992.
Laurell S, and Tibbling G. Colorimetric micro-determination of free fatty acids in plasma. Clin Chim Acta 16: 57-62, 1967.
Lawrie RA. Effect of enforced exercise on myoglobin concentration in muscle. Nature 171: 1069-1070, 1953.
Lecker SH, Goldberg AL, and Mitch WE. Protein Degradation by the Ubiquitin-Proteasome Pathway in Normal and Disease States. J Am Soc Nephrol 17: 1807-1819, 2006.
Leger B, Cartoni R, Praz M, Lamon S, Deriaz O, Crettenand A, Gobelet C, Rohmer P, Konzelmann M, Luthi F, and Russell AP. Akt signalling through GSK-3{beta}, mTOR and Foxo1 is involved in human skeletal muscle hypertrophy and atrophy. J Physiol 576: 923-933, 2006.
Legro RS, Finegood D, and Dunaif A. A Fasting Glucose to Insulin Ratio Is a Useful Measure of Insulin Sensitivity in Women with Polycystic Ovary Syndrome. J Clin Endocrinol Metab 83: 2694-2698, 1998.
Leone TC, Lehman JJ, Finck BN, Schaeffer PJ, Wende AR, Boudina S, Courtois M, Wozniak DF, Sambandam N, Bernal-Mizrachi C, Chen Z, Holloszy JO, Medeiros DM, Schmidt RE, Saffitz JE, Abel ED, Semenkovich CF, and Kelly DP. PGC-1alpha deficiency causes multi-system energy metabolic derangements: muscle dysfunction, abnormal weight control and hepatic steatosis. PLoS Biol 3: e101, 2005.
Levenhagen DK, Carr C, Carlson MG, Maron DJ, Borel MJ, and Flakoll PJ. Postexercise protein intake enhances whole-body and leg protein accretion in humans. Med Sci Sports Exerc 34: 828-837, 2002.
Levenhagen DK, Gresham JD, Carlson MG, Maron DJ, Borel MJ, and Flakoll PJ. Postexercise nutrient intake timing in humans is critical to recovery of leg glucose and protein homeostasis. Am J Physiol Endocrinol Metab 280: E982-993, 2001.
Lin J, Handschin C, and Spiegelman BM. Metabolic control through the PGC-1 family of transcription coactivators. Cell Metab 1: 361-370, 2005.
Lin J, Wu H, Tarr PT, Zhang CY, Wu Z, Boss O, Michael LF, Puigserver P, Isotani E, Olson EN, Lowell BB, Bassel-Duby R, and Spiegelman BM. Transcriptional co-

activator PGC-1 alpha drives the formation of slow-twitch muscle fibres. Nature 418: 797-801, 2002.
Lin YS, Jan MS, Tsai TJ, and Chen HI. Immunomodulatory effects of acute exercise bout in sedentary and trained rats. Med Sci Sports Exerc 27: 73-78, 1995.
Louis E, Raue U, Yang Y, Jemiolo B, and Trappe S. Time course of proteolytic, cytokine, and myostatin gene expression after acute exercise in human skeletal muscle. J Appl Physiol 103: 1744-1751, 2007.
Lowry H, and Passonneau JV. A Flexible System of Enzymatic Analysis. New York: Academic, 1972.
Lowry OH, Rosebrough NJ, Farr AL, and Randall RJ. Protein measurement with the folin phenol reagent. J Biol Chem 193: 265-275, 1951.
Luden N, Saunders M, Pratt C, Bickford A, Todd M, and Flohr J. Effects of a Six-Day Carbohyydrate/Protein Intervention on Muscle Damage, Soreness, and Performance In Runners. Med Sci Sports Exc 38: S341, 2006.
Mackinnon LT. Immunity in Athletes Int J Sports Med 18: S62 - S68, 1997.
MacNeil B, Hoffman-Goetz L, Kendall A, Houston M, and Arumugam Y. Lymphocyte proliferation responses after exercise in men: fitness, intensity, and duration effects. J Appl Physiol 70: 179-185, 1991.
Mahan MP, and Young MR. Immune parameters of untrained or exercise-trained rats after exhaustive exercise. J Appl Physiol 66: 282-287, 1989.
Manolakos J, Tang J, Kujbida G, Lysecki P, Moore D, and Phillips S. Minimal whey protein with carbohydrate stimulates muscle protein synthesis following resistance exercise in young men. FASEB J 21: LB108-a-, 2007.
Margaritis I, Tessier F, Richard MJ, and Marconnet P. No evidence of oxidative stress after a triathlon race in highly trained competitors. Int J Sports Med 18: 186-190, 1997.
Martin DE, and Hall MN. The expanding TOR signaling network. Current Opinion in Cell Biology 17: 158-166, 2005.
Martinez-Lagunas V, Ding Z, Bernard JR, Wang B, and Ivy JL. Added Protein Maintains Efficacy of a Low-Carbohydrate Sports Drink. J Strength Cond Res 2009.
Mascher H, Andersson H, Nilsson PA, Ekblom B, and Blomstrand E. Changes in signalling pathways regulating protein synthesis in human muscle in the recovery period after endurance exercise. Acta Physiol (Oxf) 191: 67-75, 2007.
McCarthy DA, and Dale MM. The leucocytosis of exercise. A review and model. Sports Med 6: 333-363, 1988.
Miller SL, Tipton KD, Chinkes DL, Wolf SE, and Wolfe RR. Independent and combined effects of amino acids and glucose after resistance exercise. Med Sci Sports Exerc 35: 449-455, 2003.
Moldoveanu AI, Shephard RJ, and Shek PN. Exercise elevates plasma levels but not gene expression of IL-1beta , IL-6, and TNF-alpha in blood mononuclear cells. J Appl Physiol 89: 1499-1504, 2000.

Morrison PJ, Hara D, Ding Z, and Ivy JL. Adding protein to a carbohydrate supplement provided after endurance exercise enhances 4E-BP1 and RPS6 signaling in skeletal muscle. J Appl Physiol 104: 1029-1036, 2008.
Morton JP, Croft L, Bartlett JD, MacLaren DPM, Reilly T, Evans L, McArdle A, and Drust B. Reduced carbohydrate availability does not modulate training-induced heat shock protein adaptations but does upregulate oxidative enzyme activity in human skeletal muscle. J Appl Physiol 106: 1513-1521, 2009.
Mougios V. Reference intervals for serum creatine kinase in athletes. Br J Sports Med 41: 674-678, 2007.
Nagashima K, Cline GW, Mack GW, Shulman GI, and Nadel ER. Intense exercise stimulates albumin synthesis in the upright posture. J Appl Physiol 88: 41-46, 2000.
Nakae J, Oki M, and Cao Y. The FoxO transcription factors and metabolic regulation. FEBS Lett 582: 54-67, 2008.
Nave BT, Ouwens M, Withers DJ, Alessi DR, and Shepherd PR. Mammalian target of rapamycin is a direct target for protein kinase B: identification of a convergence point for opposing effects of insulin and amino-acid deficiency on protein translation. Biochem J 344 Pt 2: 427-431, 1999.
Nehlsen-Cannarell SL, Nieman DC, Balk-Lamberton AJ, Markoff PA, Chritton DB, Gusewitch G, and Lee JW. The effects of moderate exercise training on immune response. Med Sci Sports Exerc 23: 64-70, 1991.
Nehlsen-Cannarella SL, Fagoaga OR, Nieman DC, Henson DA, Butterworth DE, Schmitt RL, Bailey EM, Warren BJ, Utter A, and Davis JM. Carbohydrate and the cytokine response to 2.5 h of running. J Appl Physiol 82: 1662-1667, 1997.
Newham DJ, Jones DA, and Edwards RH. Large delayed plasma creatine kinase changes after stepping exercise. Muscle Nerve 6: 380-385, 1983.
Nielson CP. Beta-adrenergic modulation of the polymorphonuclear leukocyte respiratory burst is dependent upon the mechanism of cell activation. J Immunol 139: 2392-2397, 1987.
Nieman DC. Exercise, infection, and immunity. Int J Sports Med 15 Suppl 3: S131-141, 1994.
Nieman DC. Exercise, upper respiratory tract infection, and the immune system. Med Sci Sports Exerc 26: 128-139, 1994.
Nieman DC. Immune response to heavy exertion. J Appl Physiol 82: 1385-1394, 1997.
Nieman DC. Influence of carbohydrate on the immune response to intensive, prolonged exercise. Exerc Immunol Rev 4: 64-76, 1998.
Nieman DC. Special Feature for the Olympics: Effects of Excercise on the Immune System. Immunology and Cell Biology 78: 496-501, 2000.
Nieman DC, Berk LS, Simpson-Westerber M, Arabatzis K, Youngberg S, Tan SA, Lee JW, and Eby WC. Effects of long-endurance running on immune system parameters and lymphocyte function in experienced marathoners. Int J Sports Med 10: 317-323, 1989.

Nieman DC, and Bishop NC. Nutritional strategies to counter stress to the immune system in athletes, with special reference to football. J Sports Sci 24: 763-772, 2006.
Nieman DC, Davis JM, Henson DA, Walberg-Rankin J, Shute M, Dumke CL, Utter AC, Vinci DM, Carson JA, Brown A, Lee WJ, McAnulty SR, and McAnulty LS. Carbohydrate ingestion influences skeletal muscle cytokine mRNA and plasma cytokine levels after a 3-h run. J Appl Physiol 94: 1917-1925, 2003.
Nieman DC, Fagoaga OR, Butterworth DE, Warren BJ, Utter A, Davis JM, Henson DA, and Nehlsen-Cannarella SL. Carbohydrate supplementation affects blood granulocyte and monocyte trafficking but not function after 2.5 h or running. Am J Clinical Nutrition 66: 153-159, 1997.
Nieman DC, Henson DA, Austin MD, and Brown VA. Immune response to a 30-minute walk. Med Sci Sports Exerc 37: 57-62, 2005.
Nieman DC, Henson DA, Davis JM, Angela Murphy E, Jenkins DP, Gross SJ, Carmichael MD, Quindry JC, Dumke CL, Utter AC, McAnulty SR, McAnulty LS, Triplett NT, and Mayer EP. Quercetin's influence on exercise-induced changes in plasma cytokines and muscle and leukocyte cytokine mRNA. J Appl Physiol 103: 1728-1735, 2007.
Nieman DC, Henson DA, Davis JM, Dumke CL, Utter AC, Murphy EA, Pearce S, Gojanovich G, McAnulty SR, and McAnulty LS. Blood leukocyte mRNA expression for IL-10, IL-1Ra, and IL-8, but not IL-6, increases after exercise. J Interferon Cytokine Res 26: 668-674, 2006.
Nieman DC, Henson DA, Gross SJ, Jenkins DP, Davis JM, Murphy AE, Carmichael MD, Dumke CL, Utter AC, McAnulty SR, McAnulty LS, and Mayer EP. Quercetin Reduces Illness but Not Immune Perturbations after Intensive Exercise. . Med Sci Sports Exc 39: 1561-1569, 2007.
Nieman DC, Henson DA, McAnulty SR, McAnulty LS, Morrow JD, Ahmed A, and Heward CB. Vitamin E and immunity after the Kona Triathlon World Championship. Med Sci Sports Exc 36: 1328-1335, 2004.
Nieman DC, Henson DA, Smith LL, Utter AC, Vinci DM, Davis JM, Kaminsky DE, and Shute M. Cytokine changes after a marathon race. J Appl Physiol 91: 109-114, 2001.
Nieman DC, Johanssen LM, Lee JW, and Arabatzis K. Infectious episodes in runners before and after the Los Angeles Marathon. J Sports Med Phys Fitness 30: 316-328, 1990.
Nieman DC, Miller AR, Henson DA, Warren BJ, Gusewitch G, Johnson RL, Davis JM, Butterworth DE, Herring JL, and Nehlsen-Cannarell SL. Effect of high- versus moderate-intensity exercise on lymphocyte subpopulations and proliferative response. Int J Sports Med 15: 199-206, 1994.
Nieman DC, Nehlsen-Cannarell SL, Fagoaga OR, Henson DA, Utter A, Davis JM, Williams F, and Butterworth DE. Influence of mode and carbohydrate on the cytokine response to heavy exertion. Med Sci Sports Exerc 30: 671-678, 1998.
Nieman DC, and Nehlsen-Cannarella SL. The effects of acute and chronic exercise of immunoglobulins. Sports Med 11: 183-201, 1991.

Nieman DC, Nehlsen-Cannarella SL, Fagoaga OR, Henson DA, Utter A, Davis JM, Williams F, and Butterworth DE. Effects of mode and carbohydrate on the granulocyte and monocyte response to intensive, prolonged exercise. J Appl Physiol 84: 1252-1259, 1998.
Nieman DC, and Pedersen BK. Exercise and immune function. Recent developments. Sports Med 27: 73-80, 1999.
Niles ES, Lachowetz T, Garfi J, Sullivan W, Smith JC, Leyh BP, and Headley SA. Carbohydrate-Protein Drink Improves Time to Exhaustion after Recovery from Endurance Exercise. JEPonline 4: 45-52, 2001.
Noma A, Okabe H, and Kita M. A new colorimetric micro-determination of free fatty acids in serum. Clin Chim Acta 43: 317-320, 1973.
Okazaki K, Goto M, and Nose H. Protein and carbohydrate supplementation increases aerobic and thermoregulatory capacities. The Journal of Physiology 587: 5585-5590, 2009.
Okazaki K, Ichinose T, Mitono H, Chen M, Masuki S, Endoh H, Hayase H, Doi T, and Nose H. Impact of protein and carbohydrate supplementation on plasma volume expansion and thermoregulatory adaptation by aerobic training in older men. J Appl Physiol 107: 725-733, 2009.
O'Reilly KP, Warhol MJ, Fielding RA, Frontera WR, Meredith CN, and Evans WJ. Eccentric exercise-induced muscle damage impairs muscle glycogen repletion. J Appl Physiol 63: 252-256, 1987.
Osterberg KL, Zachwieja JJ, and Smith JW. Carbohydrate and carbohydrate+protein for cycling time-trial performance. Journal of Sports Sciences 26: 227 - 233, 2008.
Ostrowski K, Hermann C, Bangash A, Schjerling P, Nielsen JN, and Pedersen BK. A trauma-like elevation of plasma cytokines in humans in response to treadmill running. J Physiol 513: 889-894, 1998.
Ostrowski K, Rohde T, Asp S, Schjerling P, and Pedersen BK. Pro- and anti-inflammatory cytokine balance in strenuous exercise in humans. J Physiol 515: 287-291, 1999.
Ostrowski K, Rohde T, Zacho M, Asp S, and Pedersen BK. Evidence that interleukin-6 is produced in human skeletal muscle during prolonged running. J Physiol 508: 949-953, 1998.
Parry-Billings M, Budgett R, Koutedakis Y, Blomstrand E, Brooks S, Williams C, Calder PC, Pilling S, Baigrie R, and Newsholme EA. Plasma amino acid concentrations in the overtraining syndrome: possible effects on the immune system. Med Sci Sports Exerc 24: 1353-1358, 1992.
Passonneau JV, and Lauderdale VR. A comparison of three methods of glycogen measurement in tissues. Anal Biochem 60: 405-412, 1974.
Pattengale PK, and Holloszy JO. Augmentation of skeletal muscle myoglobin by a program of treadmill running. Am J Physiol -- Legacy Content 213: 783-785, 1967.
Patti M-E, Brambilla E, Luzi L, Landaker EJ, and Ronald Kahn C. Bidirectional Modulation of Insulin Action by Amino Acids. J Clin Invest 101: 1519-1529, 1998.

Pedersen BK, Akerstrom TCA, Nielsen AR, and Fischer CP. Role of myokines in exercise and metabolism. J Appl Physiol 103: 1093-1098, 2007.
Pedersen BK, Bruunsgaard H, Klokker M, Kappel M, MacLean DA, Nielsen HB, Rohde T, Ullum H, and Zacho M. Exercise-induced immunomodulation--possible roles of neuroendocrine and metabolic factors. Int J Sports Med 18 Suppl 1: S2-7, 1997.
Pedersen BK, and Hoffman-Goetz L. Exercise and the Immune System: Regulation, Integration, and Adaptation. Physiol Rev 80: 1055-1081, 2000.
Pedersen BK, and Nieman DC. Exercise immunology: integration and regulation. Immunology Today 19: 204-206, 1998.
Pedersen BK, Rohde T, and Zacho M. Immunity in athletes. J Sports Med Phys Fitness 36: 236-245, 1996.
Pedersen BK, Steensberg A, and Schjerling P. Muscle-derived interleukin-6: possible biological effects. J Physiol 536: 329-337, 2001.
Pedersen BK, and Toft AD. Effects of exercise on lymphocytes and cytokines. Br J Sports Med 34: 246-251, 2000.
Pedersen BK, Tvede N, Christensen LD, Klarlund K, Kragbak S, and Halkjr-Kristensen J. Natural killer cell activity in peripheral blood of highly trained and untrained persons. Int J Sports Med 10: 129-131, 1989.
Pende M, Um SH, Mieulet V, Sticker M, Goss VL, Mestan J, Mueller M, Fumagalli S, Kozma SC, and Thomas G. S6K1-/-/S6K2-/- Mice Exhibit Perinatal Lethality and Rapamycin-Sensitive 5'-Terminal Oligopyrimidine mRNA Translation and Reveal a Mitogen-Activated Protein Kinase-Dependent S6 Kinase Pathway. Mol Cell Biol 24: 3112-3124, 2004.
Petersen AMW, and Pedersen BK. The anti-inflammatory effect of exercise. J Appl Physiol 98: 1154-1162, 2005.
Petersen EW, Carey AL, Sacchetti M, Steinberg GR, Macaulay SL, Febbraio MA, and Pedersen BK. Acute IL-6 treatment increases fatty acid turnover in elderly humans in vivo and in tissue culture in vitro. Am J Physiol Endocrinol Metab 288: E155-162, 2005.
Phillips SM, Green HJ, Tarnopolsky MA, Heigenhauser GJ, and Grant SM. Progressive effect of endurance training on metabolic adaptations in working skeletal muscle. Am J Physiol Endocrinol Metab 270: E265-272, 1996.
Phillips SM, Tipton KD, Aarsland A, Wolf SE, and Wolfe RR. Mixed muscle protein synthesis and breakdown after resistance exercise in humans. Am J Physiol Endocrinol Metab 273: E99-107, 1997.
Piehl-Aulin K, Söderlund K, and Hultman E. Muscle glycogen resynthesis rate in humans after supplementation of drinks containing carbohydrates with low and high molecular masses European Journal of Applied Physiology 81: 346-351, 2000.
Pierce AP, de Waal E, McManus LM, Shireman PK, and Chaudhuri AR. Oxidation and structural perturbation of redox-sensitive enzymes in injured skeletal muscle. Free Radic Biol Med 43: 1584-1593, 2007.

Poole DC, and Gaesser GA. Response of ventilatory and lactate thresholds to continuous and interval training. J Appl Physiol 58: 1115-1121, 1985.
Powers S, and Howley E. Exercise Physiology: Theory and Application to Fitness and Performance. New York, NY: McGraw-Hill, 2004.
Powers SK, Demirel HA, Vincent HK, Coombes JS, Naito H, Hamilton KL, Shanely RA, and Jessup J. Exercise training improves myocardial tolerance to in vivo ischemia-reperfusion in the rat. Am J Physiol Regulatory Integrative Comp Physiol 275: R1468-1477, 1998.
Powers SK, Lennon SL, Quindry J, and Mehta JL. Exercise and cardioprotection. Curr Opin Cardiol 17: 495-502, 2002.
Pratt C, Saunders M, Moore R, Hammer M, Todd M, Flohr J, and Kies A. Improved Endurance Performance With Carbohydrate- Protein Hydrolysate Beverage Not Due To Attenuated Central Fatigue. Med Sci Sports Exc 39: S362, 2007.
Pritchett K, Bishop P, Pritchett R, Green M, and Katica C. Acute effects of chocolate milk and a commercial recovery beverage on postexercise recovery indices and endurance cycling performance. Appl Physiol Nutr Metab 34: 1017-1022, 2009.
Proud CG. Regulation of mammalian translation factors by nutrients. Eur J Biochem 269: 5338-5349, 2002.
Puigserver P, and Spiegelman BM. Peroxisome Proliferator-Activated Receptor-{gamma} Coactivator 1{alpha} (PGC-1{alpha}): Transcriptional Coactivator and Metabolic Regulator. Endocr Rev 24: 78-90, 2003.
Puntschart A, Claassen H, Jostarndt K, Hoppeler H, and Billeter R. mRNAs of enzymes involved in energy metabolism and mtDNA are increased in endurance-trained athletes. Am J Physiol Cell Physiol 269: C619-625, 1995.
Rankin JW, Goldman LP, Puglisi MJ, Nickols-Richardson SM, Earthman CP, and Gwazdauskas FC. Effect of Post-Exercise Supplement Consumption on Adaptations to Resistance Training. J Am Coll Nutr 23: 322-330, 2004.
Rasmussen BB, Tipton KD, Miller SL, Wolf SE, and Wolfe RR. An oral essential amino acid-carbohydrate supplement enhances muscle protein anabolism after resistance exercise. J Appl Physiol 88: 386-392, 2000.
Raue U, Slivka D, Jemiolo B, Hollon C, and Trappe S. Proteolytic Gene Expression Differs At Rest and After Resistance Exercise Between Young and Old Women. J Gerontol A Biol Sci Med Sci 62: 1407-1412, 2007.
Rauramaa R, Halonen P, Vaisanen SB, Lakka TA, Schmidt-Trucksass A, Berg A, Penttila IM, Rankinen T, and Bouchard C. Effects of Aerobic Physical Exercise on Inflammation and Atherosclerosis in Men: The DNASCO Study: A Six-Year Randomized, Controlled Trial. Ann Intern Med 140: 1007-1014, 2004.
Reed MJ, Brozinick JT, Jr., Lee MC, and Ivy JL. Muscle glycogen storage postexercise: effect of mode of carbohydrate administration. J Appl Physiol 66: 720-726, 1989.
Reitman JS, Vasquez B, Klimes I, and Nagulesparan M. Improvement of glucose homeostasis after exercise training in non-insulin-dependent diabetes. Diabetes Care 7: 434-441, 1984.

Rhind SG, Shek PN, Shinkai S, and Shephard RJ. Effects of moderate endurance exercise and training on in vitro lymphocyte proliferation, interleukin-2 (IL-2) production, and IL-2 receptor expression. Eur J Appl Physiol Occup Physiol 74: 348-360, 1996.
Riedy M, Moore RL, and Gollnick PD. Adaptive response of hypertrophied skeletal muscle to endurance training. J Appl Physiol 59: 127-131, 1985.
Rohde T, MacLean DA, Hartkopp A, and Pedersen BK. The immune system and serum glutamine during a triathlon. Eur J Appl Physiol Occup Physiol 74: 428-434, 1996.
Romano B, Todd M, and Saunders M. Effect of a 4: 1 Ratio Carbohydrate/Protein Beverage on Endurance Performance, Muscle Damage and Recovery. Med Sci Sports Exc 36: S126, 2004.
Romano-Ely B, Todd M, Saunders M, and St. Laurent T. Effect of an Isocaloric Carbohydrate-Protein-Antioxidant Drink on Cycling Performance. Med Sci Sports Exc 38: 1608-1616, 2006.
Roux PP, and Blenis J. ERK and p38 MAPK-Activated Protein Kinases: a Family of Protein Kinases with Diverse Biological Functions. Microbiol Mol Biol Rev 68: 320-344, 2004.
Rowlands DS, Thorp RM, Rossler K, Graham DF, and Rockell MJ. Effect of protein-rich feeding on recovery after intense exercise. Int J Sport Nutr Exerc Metab 17: 521-543, 2007.
Roy BD, Tarnopolsky MA, Macdougall JD, Fowles J, and Yarasheski KE. Effect of glucose supplement timing on protein metabolism after resistance training. J Appl Physiol 82: 1882-1888, 1997.
Saltin B, Blomqvist G, JH Mitchell J, Johnson Jr R, Wildenthal K, and Chapman C. A Longitudinal Study of Adaptive Changes in Oxygen Transport and Body Composition. Circulation 38: VII 1-78, 1968.
Sarbassov DD, Guertin DA, Ali SM, and Sabatini DM. Phosphorylation and Regulation of Akt/PKB by the Rictor-mTOR Complex. Science 307: 1098-1101, 2005.
Sarbassov DD, and Sabatini DM. Redox Regulation of the Nutrient-sensitive Raptor-mTOR Pathway and Complex. J Biol Chem 280: 39505-39509, 2005.
Saunders M, Kane M, and Todd K. Effects of a Carbohydrate-Protein Beverage on Cycling Endurance and Muscle Damage. Med Sci Sports Exc 36: 1233-1238, 2004.
Saunders M, Todd M, Valentine R, St. Laurent T, Kane M, Luden N, and Herrick J. Inter-Study Examination of Physiological Variables Associated with Improved Endurance Performance with Carbohydrate/Protein Administration. Med Sci Sports Exc 38: S113-S114, 2006.
Saunders MJ, Luden ND, and Herrick JE. Consumption of an oral carbohydrate-protein gel improves cycling endurance and prevents postexercise muscle damage. J Strength Cond Res 21: 678-684, 2007.

Sawka MN, Convertino VA, Eichner ER, Schnieder SM, and Young AJ. Blood volume: importance and adaptations to exercise training, environmental stresses, and trauma/sickness. Med Sci Sports Exerc 32: 332-348, 2000.
Scharhag J, Meyer T, Auracher M, Gabriel HH, and Kindermann W. Effects of graded carbohydrate supplementation on the immune response in cycling. Med Sci Sports Exerc 38: 286-292, 2006.
Scharhag J, Meyer T, Auracher M, Muller M, Herrmann M, Gabriel H, Herrmann W, and Kindermann W. Exercise-induced increases in NT-proBNP are not related to the exercise-induced immune response. Br J Sports Med 42: 383-385, 2008.
Scholander PF. Oxygen Transport through Hemoglobin Solutions. Science 131: 585-590, 1960.
Selkirk GA, McLellan TM, Wright HE, and Rhind SG. Mild endotoxemia, NF-{kappa}B translocation, and cytokine increase during exertional heat stress in trained and untrained individuals. Am J Physiol Regulatory Integrative Comp Physiol 295: R611-623, 2008.
Shing CM, Peake J, Suzuki K, Okutsu M, Pereira R, Stevenson L, Jenkins DG, and Coombes JS. Effects of bovine colostrum supplementation on immune variables in highly trained cyclists. J Appl Physiol 102: 1113-1122, 2007.
Singh A, Ye M, Bucur O, Zhu S, Tanya Santos M, Rabinovitz I, Wei W, Gao D, Hahn WC, and Khosravi-Far R. Protein Phosphatase 2A Reactivates FOXO3a through a Dynamic Interplay with 14-3-3 and AKT. Mol Biol Cell 21: 1140-1152, 2010.
Sloan RP, Shapiro PA, DeMeersman RE, McKinley PS, Tracey KJ, Slavov I, Fang Y, and Flood PD. Aerobic exercise attenuates inducible TNF production in humans. J Appl Physiol 103: 1007-1011, 2007.
Smith LL. Cytokine hypothesis of overtraining: a physiological adaptation to excessive stress? Med Sci Sports Exerc 32: 317-331, 2000.
Sorichter S, Mair J, Koller A, Gebert W, Rama D, Calzolari C, Artner-Dworzak E, and Puschendorf B. Skeletal troponin I as a marker of exercise-induced muscle damage. J Appl Physiol 83: 1076-1082, 1997.
Sorichter S, Mair J, Koller A, Pelsers MM, Puschendorf B, and Glatz JF. Early assessment of exercise induced skeletal muscle injury using plasma fatty acid binding protein. Br J Sports Med 32: 121-124, 1998.
Spiller GA, Jensen CD, Pattison TS, Chuck CS, Whittam JH, and Scala J. Effect of protein dose on serum glucose and insulin response to sugars. Am J Clinical Nutrition 46: 474-480, 1987.
Spina RJ, Chi MM, Hopkins MG, Nemeth PM, Lowry OH, and Holloszy JO. Mitochondrial enzymes increase in muscle in response to 7-10 days of cycle exercise. J Appl Physiol 80: 2250-2254, 1996.
Sprenger H, Jacobs C, Nain M, Gressner AM, Prinz H, Wesemann W, and Gemsa D. Enhanced release of cytokines, interleukin-2 receptors, and neopterin after long-distance running. Clin Immunol Immunopathol 63: 188-195, 1992.
Srere PA. Citrate synthase. Methods Enzymol 13: 3-5, 1969.

Stitt TN, Drujan D, Clarke BA, Panaro F, Timofeyva Y, Kline WO, Gonzalez M, Yancopoulos GD, and Glass DJ. The IGF-1/PI3K/Akt pathway prevents expression of muscle atrophy-induced ubiquitin ligases by inhibiting FOXO transcription factors. Mol Cell 14: 395-403, 2004.
Suzuki K, Naganuma S, Totsuka M, Suzuki KJ, Mochizuki M, Shiraishi M, Nakaji S, and Sugawara K. Effects of exhaustive endurance exercise and its one-week daily repetition on neutrophil count and functional status in untrained men. Int J Sports Med 17: 205-212, 1996.
Suzuki K, Nakaji S, Yamada M, Totsuka M, Sato K, and Sugawara K. Systemic inflammatory response to exhaustive exercise. Cytokine kinetics. Exerc Immunol Rev 8: 6-48, 2002.
Suzuki K, Sato H, Kikuchi T, Abe T, Nakaji S, Sugawara K, Totsuka M, Sato K, and Yamaya K. Capacity of circulating neutrophils to produce reactive oxygen species after exhaustive exercise. J Appl Physiol 81: 1213-1222, 1996.
Suzuki K, Totsuka M, Nakaji S, Yamada M, Kudoh S, Liu Q, Sugawara K, Yamaya K, and Sato K. Endurance exercise causes interaction among stress hormones, cytokines, neutrophil dynamics, and muscle damage. J Appl Physiol 87: 1360-1367, 1999.
Suzuki M. Glycemic carbohydrates consumed with amino acids or protein right after exercise enhance muscle formation. Nutr Rev 61: S88-94, 2003.
Taillandier D, Aurousseau E, Meynial-Denis D, Bechet D, Ferrara M, Cottin P, Ducastaing A, Bigard X, Guezennec CY, and Schmid HP. Coordinate activation of lysosomal, Ca 2+-activated and ATP-ubiquitin-dependent proteinases in the unweighted rat soleus muscle. Biochem J 316 (Pt 1): 65-72, 1996.
Tarnopolsky MA, Bosman M, Macdonald JR, Vandeputte D, Martin J, and Roy BD. Postexercise protein-carbohydrate and carbohydrate supplements increase muscle glycogen in men and women. J Appl Physiol 83: 1877-1883, 1997.
Terada S, Goto M, Kato M, Kawanaka K, Shimokawa T, and Tabata I. Effects of low-intensity prolonged exercise on PGC-1 mRNA expression in rat epitrochlearis muscle. Biochem Biophys Res Commun 296: 350-354, 2002.
Thomas K, Morris P, and Stevenson E. Improved endurance capacity following chocolate milk consumption compared with 2 commercially available sport drinks. Appl Physiol Nutr Metab 34: 78-82, 2009.
Tipton KD, Elliott TA, Cree MG, Wolf SE, Sanford AP, and Wolfe RR. Ingestion of casein and whey proteins result in muscle anabolism after resistance exercise. Med Sci Sports Exerc 36: 2073-2081, 2004.
Tipton KD, Ferrando AA, Phillips SM, Doyle D, Jr., and Wolfe RR. Postexercise net protein synthesis in human muscle from orally administered amino acids. Am J Physiol Endocrinol Metab 276: E628-634, 1999.
Toft AD, Jensen LB, Bruunsgaard H, Ibfelt T, Halkjar-Kristensen J, Febbraio M, and Pedersen BK. Cytokine response to eccentric exercise in young and elderly humans. Am J Physiol Cell Physiol 283: C289-295, 2002.

Totsuka M, Naganuma S, Suzuki K, Nakaji S, Sato S, and Sugawara S. Rapid adaptation of creatine kinase responses to repeated daily endurance exercise. J Phys Fitness Nutr Immunol 6: 187-190, 1996.
Totsuka M, Nakaji S, Suzuki K, Sugawara K, and Sato K. Break point of serum creatine kinase release after endurance exercise. J Appl Physiol 93: 1280-1286, 2002.
Ueki K, Yamamoto-Honda R, Kaburagi Y, Yamauchi T, Tobe K, Burgering BMT, Coffer PJ, Komuro I, Akanuma Y, Yazaki Y, and Kadowaki T. Potential Role of Protein Kinase B in Insulin-induced Glucose Transport, Glycogen Synthesis, and Protein Synthesis. J Biol Chem 273: 5315-5322, 1998.
Ullum H, Haahr PM, Diamant M, Palmo J, Halkjaer-Kristensen J, and Pedersen BK. Bicycle exercise enhances plasma IL-6 but does not change IL-1 alpha, IL-1 beta, IL-6, or TNF-alpha pre-mRNA in BMNC. J Appl Physiol 77: 93-97, 1994.
Valentine RJ, Saunders MJ, Todd MK, and St Laurent TG. Influence of carbohydrate-protein beverage on cycling endurance and indices of muscle disruption. Int J Sport Nutr Exerc Metab 18: 363-378, 2008.
van Essen M, and Gibala MJ. Failure of protein to improve time trial performance when added to a sports drink. Med Sci Sports Exerc 38: 1476-1483, 2006.
van Hall G, Shirreffs SM, and Calbet JAL. Muscle glycogen resynthesis during recovery from cycle exercise: no effect of additional protein ingestion. J Appl Physiol 88: 1631-1636, 2000.
van Hall G, Steensberg A, Sacchetti M, Fischer C, Keller C, Schjerling P, Hiscock N, Moller K, Saltin B, Febbraio MA, and Pedersen BK. Interleukin-6 Stimulates Lipolysis and Fat Oxidation in Humans. J Clin Endocrinol Metab 88: 3005-3010, 2003.
van Loon LJC, Saris WHM, Kruijshoop M, and Wagenmakers AJM. Maximizing postexercise muscle glycogen synthesis: carbohydrate supplementation and the application of amino acid or protein hydrolysate mixtures. Am J Clinical Nutrition 72: 106-111, 2000.
Wagenmakers AJ, Brouns F, Saris WH, and Halliday D. Oxidation rates of orally ingested carbohydrates during prolonged exercise in men. J Appl Physiol 75: 2774-2780, 1993.
Wallis GA, Rowlands DS, Shaw C, Jentjens RL, and Jeukendrup AE. Oxidation of combined ingestion of maltodextrins and fructose during exercise. Med Sci Sports Exerc 37: 426-432, 2005.
Walsh NP, Blannin AK, Bishop NC, Robson PJ, and Gleeson M. Effect of oral glutamine supplementation on human neutrophil lipopolysaccharide-stimulated degranulation following prolonged exercise. Int J Sport Nutr Exerc Metab 10: 39-50, 2000.
Walsh NP, Blannin AK, Robson PJ, and Gleeson M. Glutamine, exercise and immune function. Links and possible mechanisms. Sports Med 26: 177-191, 1998.
Wang L, Rhodes CJ, and Lawrence JC, Jr. Activation of Mammalian Target of Rapamycin (mTOR) by Insulin Is Associated with Stimulation of 4EBP1 Binding to Dimeric mTOR Complex 1. J Biol Chem 281: 24293-24303, 2006.

Wang X, Paulin FE, Campbell LE, Gomez E, O'Brien K, Morrice N, and Proud CG. Eukaryotic initiation factor 2B: identification of multiple phosphorylation sites in the epsilon-subunit and their functions in vivo. EMBO J 20: 4349-4359, 2001.
Wang X, and Proud CG. A Novel Mechanism for the Control of Translation Initiation by Amino Acids, Mediated by Phosphorylation of Eukaryotic Initiation Factor 2B. Mol Cell Biol 28: 1429-1442, 2008.
Weiland O. Glycerol, UV-method. In: Methods of enzymatic analysis, edited by Bergmeyer HU. New York: Academic Press, 1974, p. 1404–1409.
Wilkinson SB, Tarnopolsky MA, MacDonald MJ, MacDonald JR, Armstrong D, and Phillips SM. Consumption of fluid skim milk promotes greater muscle protein accretion after resistance exercise than does consumption of an isonitrogenous and isoenergetic soy-protein beverage. Am J Clinical Nutrition 85: 1031-1040, 2007.
Williams MB, Raven PB, Fogt DL, and Ivy JL. Effects of Recovery Beverages on Glycogen Restoration and Endurance Exercise Performance. Journal of Strength and Conditioning Research 17: 12-19, 2003.
Williamson DL, Kubica N, Kimball SR, and Jefferson LS. Exercise-induced alterations in extracellular signal-regulated kinase 1/2 and mammalian target of rapamycin (mTOR) signalling to regulatory mechanisms of mRNA translation in mouse muscle. J Physiol 573: 497-510, 2006.
Williamson DL, Raue U, Slivka DR, and Trappe S. Resistance Exercise, Skeletal Muscle FOXO3A, and 85-Year-Old Women. J Gerontol A Biol Sci Med Sci 65A: 335-343, 2010.
Wojcik JR, Walber-Rankin J, Smith LL, and Gwazdauskas FC. Comparison of carbohydrate and milk-based beverages on muscle damage and glycogen following exercise. Int J Sport Nutr Exerc Metab 11: 406-419, 2001.
Wright DC, Han D-H, Garcia-Roves PM, Geiger PC, Jones TE, and Holloszy JO. Exercise-induced Mitochondrial Biogenesis Begins before the Increase in Muscle PGC-1{alpha} Expression. J Biol Chem 282: 194-199, 2007.
Wu Z, Puigserver P, Andersson U, Zhang C, Adelmant G, Mootha V, Troy A, Cinti S, Lowell B, Scarpulla RC, and Spiegelman BM. Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. Cell 98: 115-124, 1999.
Yang RC, Mack GW, Wolfe RR, and Nadel ER. Albumin synthesis after intense intermittent exercise in human subjects. J Appl Physiol 84: 584-592, 1998.
Yaspelkis BB, 3rd, and Ivy JL. Effect of carbohydrate supplements and water on exercise metabolism in the heat. J Appl Physiol 71: 680-687, 1991.
Yaspelkis BB, 3rd, Patterson JG, Anderla PA, Ding Z, and Ivy JL. Carbohydrate supplementation spares muscle glycogen during variable-intensity exercise. J Appl Physiol 75: 1477-1485, 1993.
Zaldivar F, Wang-Rodriguez J, Nemet D, Schwindt C, Galassetti P, Mills PJ, Wilson LD, and Cooper DM. Constitutive pro- and anti-inflammatory cytokine and growth factor response to exercise in leukocytes. J Appl Physiol 100: 1124-1133, 2006.

Zawadzki KM, Yaspelkis BB, 3rd, and Ivy JL. Carbohydrate-protein complex increases the rate of muscle glycogen storage after exercise. J Appl Physiol 72: 1854-1859, 1992.
Zemel MB. Mechanisms of Dairy Modulation of Adiposity. J Nutr 133: 252S-256, 2003.
Zemel MB. Role of calcium and dairy products in energy partitioning and weight management. Am J Clinical Nutrition 79: 907S-912, 2004.